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Transition to an alternate state in a continental shelf ecosystem

Jae S. Choi, Kenneth T. Frank, William C. Leggett, and Ken Drinkwater

Abstract: The collapse of Atlantic cod (*Gadus morhua*) stocks in the northwest Atlantic Ocean ranks among the most dramatic and widely known ecological changes of the 20th century. Less widely known are the systemic changes that occurred within these areas prior to and coincident with the cod collapse. Our analysis of a fishery-independent, long-term, standardized database collected on the eastern Scotian Shelf off Nova Scotia revealed that during the past four decades, coherent, community-level reductions in body size, biomass, and physiological condition have occurred in the resident demersal fish species. The changes occurred over large spatial ($>10^4$ km^2) and short temporal (<10 years) scales, suggesting a progressive decline in the nature and extent of the energy flow through the benthic system. The unexpected and persistent poor condition of a variety of morphologically and functionally dissimilar demersal fish species, living in an environment of reduced intra- and inter-specific competition, suggests a decoupling of the benthic–pelagic systems. This decoupling appears to be attributable to a cascading series of processes involving the cumulative removal of biomass resulting from commercial fishing, compensatory and self-stabilizing increases in pelagic fish biomass, and a decline in groundfish productivity exacerbated by decadal scale variability in water temperature and stratification.

Résumé : L'effondrement des stocks de morues de l'Atlantique (*Gadus morhua*) dans le nord-ouest de l'océan Atlantique constitue l'un des changements écologiques les plus spectaculaires et les mieux connus du 20$^{\text{ième}}$ siècle. Ce qui demeure moins bien documenté, ce sont les changements systémiques qui sont survenus dans ces régions avant et pendant l'effondrement des populations de morues. Notre analyse d'une banque de données standardisées et récoltées sur une longue période indépendamment de la pêche commerciale sur l'est de la plate-forme Néo-écossaise au large de la Nouvelle-Écosse révèle qu'au cours des quatre dernières décennies, il s'est produit au niveau de la communauté des réductions coordonnées de la taille corporelle, de la biomasse et de la condition physiologique chez les espèces locales de poissons démersaux. Ces changements ont eu lieu sur une vaste échelle spatiale ($>10^4$ km^2) et une courte échelle temporelle (<10 ans), ce qui laisse croire à un déclin progressif de la nature et de l'importance du flux d'énergie dans le système benthique. La mauvaise condition physiologique, à la fois imprévue et persistante, d'une variété d'espèces de poissons démersaux à morphologie et biologie différentes qui vivent dans un milieu de faible compétition intra- et inter-spécifique semble indiquer une dissociation entre les systèmes benthique et pélagique. Ce découplage paraît être causé par une série de processus en cascade reliés au retrait cumulatif de la biomasse par la pêche commerciale, à l'augmentation par auto-stabilisation de la biomasse des poissons pélagiques et au déclin de la productivité des poissons de fond, le tout aggravé par une variabilité à l'échelle de la décennie de la température et de la stratification de l'eau.

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Introduction

In 1993, after centuries of commercial fishing, the once extensive fishery for cod (*Gadus morhua*) on the eastern Scotian Shelf off the coast of Nova Scotia, Canada, collapsed and a moratorium on directed fishing was declared. A decade later, with this management measure still in effect, there is no evidence of recovery. The spectacular nature of this collapse and the scientific and public attention that it received has obscured the much more profound coincident ecological collapse of an entire complex of previously abundant demersal fishes, many of which were subjected to only modest exploitation. These rapid, large-scale changes suggest the involvement of broader ecosystem-level influences. In this paper we explore and describe the structural and functional changes that have occurred in the fish community and use them as indicators of ecological change. We then speculate on the probable causes of this catastrophic decline of the formerly dominant demersal community and transition of the system to an alternate state.

Methods

Our evaluation relied heavily upon research surveys of commercial and noncommercial fish species resident on the eastern half of the Scotian Shelf off Nova Scotia. This widely used, randomized, depth-stratified summer survey consists of an average of 100 sets each year and has been conducted annually by the Canadian Department of Fisheries and Oceans from 1970 to present. Although designed to capture bottom-dwelling species (mainly groundfish), other species such as small pelagics are routinely captured (Table 1). The catch for each set is sorted by species, enumerated, and weighed. Additional measurements include individual length and weight for most of the species. These data were used to compute body size and biomass trends over time. No changes have been made to the survey design, although a few changes associated with gear and vessel have occurred. Gear type changed once from the Yankee 36 to the Western IIA in 1982 and vessel changes occurred between 1981 and 1983. Comparative fishing experiments yielded catchability corrections for cod, haddock (*Melanogrammus aeglefinus*), witch flounder (*Glyptocephalus cynoglossus*), yellowtail flounder (*Limanda ferruginea*), winter flounder (*Pseudopleuronectes americanus*), American plaice (*Hippoglossoides platessoides*), silver hake (*Merluccius bilinearis*), and white hake (*Urophycis tenuis*), which have been applied to the data for 1970 to 1981.

Species composition was assessed using correspondence analysis, a robust multivariate ordination technique, in a spatially explicit manner. The numerical abundance per unit effort at each sampling location was used, after $\log_{10}(n + 1)$ transformation to normalize the data. Significance levels for Pearson product–moment correlations were not adjusted for temporal autocorrelation.

Individual fish lengths and weights were used to compute indices of physiological condition. Condition was expressed as the probability value associated with the average standardized residual mass of a given set location using the Z-score transform (residuals were normally distributed). The residuals were determined from unique $\log_{10}$(length) versus $\log_{10}$(weight) linear regression models established for each of the species. Length and weight measurements were available for the dominant species only, which amounted to 53 species. Low probability values (e.g., <0.05) indicate that a community's physiological condition is lower than normal and high probability values (e.g., >0.95) represent communities with higher than normal physiological condition. The data set was complete with the exception that during 1986 to 1994, the lengths and weights of only the six most important commercial species (cod, haddock, pollock (*Pollachius virens*), silver hake, redfish (*Sebastes* spp.), American plaice) were recorded (Table 1). All data analysis was conducted with *R* (http://www.R-project.org). Data were mapped after discretization to a 2-min grid size and spatially interpolated within a 40-km radius using the continuous curvature "splines-in-tension" algorithm in GMT (http://gmt.soest.hawaii.edu; tension = 0.2).

Results and discussion

Structural changes

Body size

Spatially coherent, within- and between-species reductions of mean body size were observed (Fig. 1*a*). Throughout the 1970s and 1980s, large individuals dominated the northeastern half of the shelf. Individuals >8 kg were found in >75% of the area sampled and by the 1990s had declined to 40%. Currently large fish occur on only 10% of the area surveyed and are restricted to the northeastern edge of the shelf. Numerically, the magnitude of this change was profound. Relative to the 1970s, there are currently 95% fewer fish >8 kg and fish of intermediate size (1–8 kg) are 60% lower in abundance. Conversely, small fish (<0.1 kg and 0.1–1 kg) have increased by 375% and 100%, respectively. These changes in body size result from variations in species composition, size-specific mortality, and reduced growth rates (Department of Fisheries and Oceans (DFO) 2003).

Biomass

Fish biomass was partitioned between groundfish and pelagic species (Figs. 1*b*, 1*c*). Historically, groundfish biomass dominated the Scotian Shelf ecosystem (Zwanenburg et al. 2002). Here too, the changes were profound. In the central portion of the shelf, groundfish biomass per standard tow progressively declined throughout the 1970s and 1980s and by the 1990s was two orders of magnitude lower relative to the 1970s. The areal extent of biomass >100 kg per tow decreased from 32% and 49% in the 1970s and 1980s, respectively, to <1% in the 1990s and 2000s. This decline was mainly associated with the collapse of cod and haddock but was also accompanied by reductions of other less heavily exploited commercial species (Table 1). Biomass levels remained relatively high in only two areas: adjacent to the central region, where a large (14 000 km^2) marine protected area was established in 1987, and on the slope along the northeastern edge of the shelf.

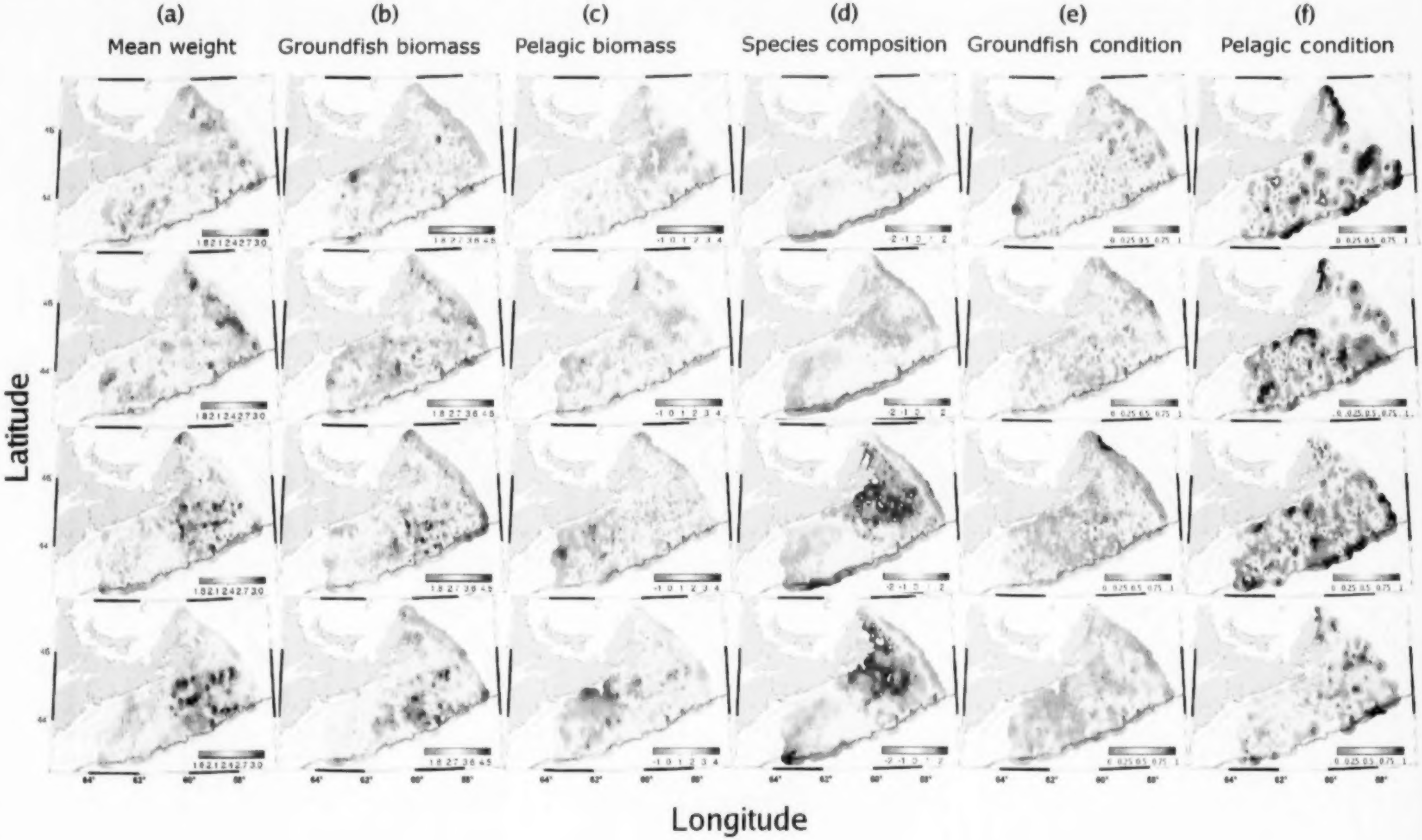
Pelagic biomass was uniformly low during the 1970s and 1980s. In no area did the biomass of pelagic fish species exceed 10 kg per standard tow. By the 2000s, catches containing pelagic biomass >10 kg per standard tow had increased

Table 1. List of fish species used in the analysis of condition.

Scientific name	Common name	C/NC	D/P	Biomass	*r*	*n*	*p* value
Centroscyllium fabricii	Black dogfish	0	0	19.23	–0.73	3	0.48
Lycodes lavalaei	Laval's eelpout	0	0	17.03	–0.70	4	0.30
Melanogrammus aegelfinus	Haddock	1	0	1098.68	–0.66	34	<0.001
Cryptacanthodes maculatus	Wrymouth	0	0	17.18	–0.62	7	0.14
Gadus morthua	Cod	1	0	815.69	–0.55	34	<0.001
Merluccius bilinearis	Silver hake	1	0	404.37	–0.51	34	<0.001
Scophthalmus aquosus	Brill/windowpane	1	0	23.59	–0.51	10	0.14
Merluccius albidus	Offshore hake	0	0	66.99	–0.49	17	0.05
Lycodes reticulatus	Arctic eelpout	0	0	32.24	–0.48	7	0.27
Tautogolabrus adspersus	Cunner	0	0	37.69	–0.48	6	0.34
Urophycis chuss	Red hake	1	0	47.79	–0.47	20	0.04
Peprilus triacanthus	Butterfish	0	1	22.48	–0.46	8	0.25
Sebastes sp.	Redfish	1	0	821.14	–0.46	32	0.01
Pseudopleuronectes americanus	Winter flounder	1	0	154.09	–0.45	25	0.02
Raja erinacea	Little skate	0	0	88.94	–0.40	14	0.15
Cyclopterus lumpus	Lumpfish	0	0	66.00	–0.39	7	0.38
Macrozoarces americanus	Ocean pout	0	0	30.41	–0.39	23	0.07
Phycis chesteri	Longfin hake	0	0	43.08	–0.36	24	0.08
Hippoglossus hippoglossus	Atlantic halibut	1	0	130.51	–0.32	26	0.11
Raja ocellata	Winter skate	1	0	319.96	–0.32	24	0.13
Glyptocephalus cynoglossus	Witch flounder	1	0	48.13	–0.31	26	0.13
Lycodes terraenova	Eelpout (Nfld.)	0	0	19.79	–0.27	5	0.67
Limanda ferruginea	Yellowtail flounder	1	0	283.66	–0.26	25	0.20
Hippoglossoides platessoides	American plaice	1	0	298.66	–0.26	34	0.14
Lophius americanus	Monkfish	1	0	126.76	–0.22	25	0.29
Agonus decagonus	Sea poacher	0	0	1.1	–0.20	9	0.60
Helicolenus dactylopterus	Blackbelly rosefish	0	0	5.88	–0.20	3	0.87
Hemitripterus americanus	Sea raven	0	0	59.3	–0.17	23	0.43
Argentina silus	Argentine	0	1	66.51	–0.16	23	0.45
Pollachius virens	Pollock	1	0	588.37	–0.14	34	0.44
Lycodes vahlii	Shorttailed eelpout	0	0	26.73	–0.13	18	0.62
Notolepis rissoi kroyeri	White barracudina	0	0	3.00	–0.10	8	0.81
Myoxocephalus octodecemspinosus	Longhorn sculpin	0	0	76.1	–0.05	24	0.82
Myoxocephalus scorpius	Shorthorn sculpin	0	0	17.02	–0.05	7	0.92
Squalus acanthias	Spiny dogfish	1	0	355.91	–0.01	18	0.98
Scomber scombrus	Mackerel	1	1	159.57	0.04	23	0.87
Myxine glutinosa	Northern hagfish	0	0	10.39	0.06	17	0.81
Raja senta	Smooth skate	0	0	20.17	0.13	24	0.53
Urophycis tenuis	White hake	1	0	203.44	0.17	25	0.42
Clupea harengus	Herring	1	1	552.94	0.18	22	0.42
Enchelyopus cimbrius	Fourbeard rockling	0	0	2.50	0.19	20	0.41
Anarhichas denticulatus	Northern wolffish	1	0	191.42	0.22	4	0.78
Nezumia bairdi	Marlinspike grenadier	0	0	8.02	0.24	24	0.25
Anarhichas lupus	Striped Atlantic wolffish	1	0	103.17	0.25	23	0.25
Brosme brosme	Cusk	1	0	128.38	0.26	19	0.28
Raja radiata	Thorny skate	1	0	159.64	0.27	25	0.19
Reinhardtius hippoglossoides	Greenland halibut	1	0	73.39	0.39	24	0.06
Lycodes sp.	Eelpouts	0	0	70.33	0.53	7	0.22
Lumpenus lumpretaeformis	Snake blenny	0	0	5.29	0.88	10	<0.001
Raja laevis	Barndoor skate	0	0	535.79	0.92	4	0.08
Alosa sapidissima	American shad	0	1	31.86	0.97	3	0.15
Paralichthys oblongus	Fourspot flounder	0	0	18.59	1.00	3	0.03
Mallotus villosus	Capelin	0	1	31.86	—	*	—
Alosa pseudoharengus	Alewife	0	1	10.25	—	*	—
Anarhichas minor	Spotted wolffish	1	0	126.45	—	2	—

Note: Species are listed as being either commercial, C (1), or noncommercial, NC (0), and pelagic, P (1), or demersal, D (0). Geometric mean biomass ($kg \cdot km^{-2}$) from 1970 to 2003 is also shown, as well as the temporal correlation of condition (*r*), associated *p* values, and sample size for each species (*n*). The species list has been sorted by the sign and strength of the Pearson correlation coefficient. Variable sample sizes are due to changing protocols, absence of species in some years, or a cutoff criterion imposed on fish less than 20 cm because of the unreliability of weight measurements at these smaller sizes that occurred during the 1970s and 1980s (denoted by an asterisk (*)). Most demersal species exhibited negative temporal trends in condition with about a dozen species showing relatively strong trends.

Fig. 1. Spatiotemporal depictions of selected metrics of the eastern Scotian Shelf fish community. (Column *a*) Geometric mean weight of all groundfish species for each decade expressed in units of $\log_{10}$(weight; g). Note the dramatic reduction of body size throughout the eastern Scotian Shelf. (Columns *b* and *c*) Geometric mean biomass per standard tow of groundfish and pelagic fish species in units of $\log_{10}$(biomass; kg). Biomass of groundfish and pelagic fish has been declining throughout the region with the exception of the western side where pelagic biomass has recently increased. (Column *d*) Species composition expressed as the second axis of variation in a correspondence analysis. The first axis of variation accounted for 7.0% of the total variance and was associated with a depth gradient (i.e., contrasting deep-water to shallow-water assemblages). The second axis of variation was associated with bottom temperatures, accounting for 6.8% of the total variation. (Columns *e* and *f*) Community condition index of groundfish and pelagic fish species expressed as the significance level of the mean standardized residual weight of all individuals sampled in a survey. Note the erosion of physiological condition of the majority of groundfish and the increase in condition of pelagic fish species over the last four decades.


(a)
Mean weight
(b)
Groundfish biomass
(c)
Pelagic biomass
(d)
Species composition
(e)
Groundfish condition
(f)
Pelagic condition
1970s
1980s
1990s
2000s
Latitude
Longitude
46
44
64°
62°
60°
58°
1.8 2.1 2.4 2.7 3.0
1.8 2.7 3.6 4.5
-1 0 1 2 3 4
-2 -1 0 1 2
0 0.25 0.5 0.75 1

to 23% of the total area. The greatest increases in pelagic catches occurred in the west and in a few isolated regions to the east. Current pelagic biomass estimates are approximately two orders of magnitude higher than those of the 1970s. This is attributable primarily to the increased abundance of herring (*Clupea harengus*) in the west, capelin (*Mallotus villosus*) in the east, and sandlance (*Ammodytes dubius*) throughout. These increases in abundance were accompanied by the development of a large offshore herring fishery on the outer banks of the central Scotian Shelf where none had existed before 1996 (DFO 2003).

Increases in pelagic biomass are, in part, related to predation release (Swain and Sinclair 2000), and their reciprocal abundance patterns ($r_{\text{Pearson}} = -0.55$, df = 31, $p < 0.001$) support this conclusion. However, none of the groundfish species studied is strictly piscivorous, with most feeding on a wide variety of benthic macroinvertebrates (Garrison and Link 2000). This fact probably accounts for the high residual variance in the relationship. Comparative studies have also shown that in temperate marine fish communities, pelagic species tend to proliferate in areas where water column stratification is stronger (Steele and Schumacher 2000). Temporal increases in stratification intensity were positively correlated with pelagic biomass ($r_{\text{Pearson}} = 0.50$, df = 31, $p < 0.001$). Also, the increased capacity of the pelagic community to compete and prey upon groundfish, particularly their early life stages, which themselves tend to also be pelagic, may also have contributed to their increased relative dominance in the region. This autocatalytic positive-feedback mechanism in which pelagic fish regulate the recruitment of their former predators has been documented elsewhere (Swain and Sinclair 2000).

Species composition

A spatially explicit, multivariate ordination of species abundances revealed dramatic changes in community structure (Fig. 1*d*). First, the ordination revealed a distinct temporal trend towards increasing dominance of shallow-water species (principally pelagic species). Second, species new to the area associated with variations in bottom temperatures were evident. This latter gradient was portrayed spatially; during the 1970s and 1980s, community composition was relatively stable, the eastern half of the region being distinct (colder-water species) from the west (warmer-water species). By the 1990s, the eastern region had become dominated by smaller-bodied species of subarctic origin. Only minor changes were observed to the west.

Functional changes

Condition

During the 1970s, aggregate "condition" was higher than normal in 44% of the survey area. By the 1980s, this value had plummeted to 7% and continued to decline in the 1990s to 3% and finally to 2% by the 2000s, reflecting a sustained reduction in physiological condition (Fig. 1*e*). The most dramatic reductions occurred in the western half of the region where the community composition remained relatively stable. Collectively, these patterns reveal progressive erosion in the bioenergetic "well-being" of the resident groundfish community. The species that exhibited the strongest trends in condition were quite diverse in terms of their morphology, dietary preferences, and general life history characteristics (Table 1). Only in the east where species composition became dominated by invading subarctic species associated with the incursion of colder water did the condition index remain greater than normal.

Aggregate condition of pelagic species (Table 1) was variable throughout the survey area: the estimated surface area occupied by pelagics with greater than normal condition changed from 34%, 27%, 14%, and 42% during the 1970s, 1980s, 1990s, and 2000s, respectively (Fig. 1*f*). The spatial and temporal patterns contrast sharply with those observed in the condition of groundfish.

Speculation on the causes of the transition

Why was there a widespread occurrence of a diverse assemblage of groundfish species in poor condition at a time (mid-1980s to 2000s) when intra- and inter-specific competition for food resources was expected to be low? We consider one of the leading hypotheses to explain this poor health of the resident groundfish to be energy depletion in the system. Two primary factors causing this energy depletion involve cumulative biomass removal and abiotic change.

The removal of biomass from the eastern Scotian Shelf ecosystem as a result of fishing was enormous. Since 1960 (i.e., ignoring all previous human exploitation), fishing removed 9000 kt of fish and macroinvertebrates without replacement (of which groundfish represented ~80%). A conservative estimate of the "net primary production required" to accommodate or sustain this removal of groundfish biomass is of the order of 200 $g\ C\cdot m^{-2}\cdot year^{-1}$ (assuming a trophic transfer efficiency of 10% and three feeding steps, as per Pauly and Christensen 1995). This value is of the same order of magnitude as the net primary production observed in many temperate continental shelf systems (Nixon 1988) and that of the Scotian Shelf (<100 $g\ C\cdot m^{-2}\cdot year^{-1}$; Dauchez et al. 1996). To compensate for the biologically useful biomass removed on the Scotian Shelf would require that every unit of net primary production be shunted into groundfish biomass production, and likely much more. This by-product of benthic biomass removal may, in turn, have contributed to and accelerated the decline in the dominance of the groundfish.

Although some possibly compensatory increases in the abundance of exploited benthic macroinvertebrates (shrimp (*Pandalus borealis*) and snow crab (*Chionoecetes opilio*)) have been observed (current biomass estimate of 60 kt), such increases are still low relative to the peak groundfish abundance in the 1970s and 1980s (DFO 2003). Insufficient data exist to evaluate the abundance of other benthic invertebrate species and it is therefore highly uncertain whether or not the benthic system is energy poor. However, the prolonged period of low and declining condition reflected in dozens of diverse groundfish species is indicative of limited abundance of a broad spectrum of benthic prey types (Fig. 1*d*).

Abiotic conditions known to influence ecosystem productivity underwent major changes coincident with the community and population level changes observed on the eastern Scotian Shelf. The kinetic energy regime on the eastern Scotian Shelf has been dominated since 1987 by increased density stratification in the top 50 m of the water column (primarily due to surface advection of low-salinity waters from the north; Drinkwater et al. 2003). This diminished the intermixing of surface and deep water layers and is likely to have reduced the exchange of limiting nutrients between them (i.e., new production). It is noteworthy that current rates of ocean primary production are estimated to have declined ~6.7% in the North Atlantic based on remote sensing data, relative to the early 1980s (Gregg et al. 2003). This would, in turn, reduce the degree to which the pelagic ecosystem acts as an engine of energy production for the benthic community. In addition, there was an increased presence of cold subsurface waters in the region. Bottom temperatures declined throughout the 1980s, dropped below the long-term mean in 1985, and reached a new sustained minimum in the early 1990s. This 15+-year period of colder than normal temperatures is unprecedented in the 50+-year temperature record for the region (Drinkwater et al. 2003). The consequences of these temperature changes are particularly limiting for the growth and recruitment of the component populations of the demersal fish community whose habitat is, by definition, largely restricted to the bottom. Pelagic fishes, in contrast, are more flexible in their capacity to choose and so optimize their use of temperature gradients and resources throughout the water column thereby reducing the influence of such changes.

Future outlook

A convergence of individuals within the groundfish community of the Scotian Shelf to a narrow range of small body sizes has occurred over the past 30+ years. This suggests a functional bottlenecking among species unlike anything recorded in the past leading to (*i*) a shortening of the characteristic time scales of the population dynamic processes relevant to the groundfish (growth, reproduction, mortality) and (*ii*) a less energetically efficient system as mass-specific heat production increases as organism size decreases as a consequence of the allometry of biological rate processes (Peters 1983). These characteristics, reflected in the changes seen in the groundfish community of the eastern Scotian Shelf, are indicative of a severely perturbed system with reduced functional diversity (Choi and Patten 2001). Clearly a hysteresis has occurred in the Scotian Shelf ecosystem. This makes the prediction of the conditions required for stock recovery and their associated time scales difficult and highly uncertain. The complexity of the interacting factors involved in the dramatic restructuring of the Scotian Shelf ecosystem highlights the problems inherent in attempting to manage individual species within the framework of a complex and dynamic ecosystem.

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Lack of long-term sublethal effects of reduced freshwater pH alone on Atlantic salmon (*Salmo salar*) smolts subsequently transferred to seawater

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Abstract: Atlantic salmon (*Salmo salar*) smolts (mean start weight 42 g) in soft freshwater were exposed to three pH ranges (two replicates in each group) for 35 days: pH 6.5–6.8 (control group), pH 5.9–6.3 (medium-pH group), and pH 5.4–5.9 (low-pH group). All exposures had citrate added to remove labile, toxic Al from the water. On day 35, all groups were transferred to 34‰ seawater and kept there for 100 days. H^+ was the main stressor in the exposures because labile Al was <6 $\mu g \cdot L^{-1}$ and the gill Al was lower than 27 $\mu g \cdot g$ dry weight^{-1} (highest in the medium group). The exposure environments did not cause any significant changes to gill tissue structures, gill Na^+,-K^+-ATPase activity, mortality, and growth parameters during the freshwater period. However, haematocrit was significantly increased and mean plasma chloride was significantly reduced ($p < 0.05$) in the low-pH group compared with the control group. This study indicated that the H^+ concentrations in the pH range 5.4–5.9 in water containing no to little gill-reactive Al do not impact salmon growth and physiology during smoltification. However, the reduction in blood haematocrit in the low-pH group 3 months after seawater transfer may imply long-term effects of the treatment.

Résumé : Des saumoneaux du saumon de l'Atlantique (*Salmo salar*), de masse moyenne de 42 g au départ, gardés en eau douce de faible dureté ont été exposés à trois gammes de pH (deux expériences dans chaque groupe) pendant 35 jours, soit à pH 6,5–6,8 (groupe témoin), pH 5,9–6,3 (groupe à pH moyen) et à pH 5,4–5,9 (groupe à pH bas). Du citrate a été ajouté dans toutes les expériences pour retirer l'aluminium (Al) labile et toxique de l'eau. Au jour 35, tous les groupes ont été transférés en eau de mer 34 ‰ et gardés là pendant 100 jours. H^+ était le facteur principal de stress dans les expériences puisque la concentration d'Al labile était <6 $\mu g \cdot L^{-1}$ et que l'Al dans les branchies ne dépassait pas 27 μg/g de masse sèche (maximum dans le groupe moyen). Les milieux expérimentaux n'ont causé de changements significatifs ni de la structure du tissu branchial, ni de l'activité de la Na^+,-K^+-ATPase de la branchie, ni des paramètres de mortalité et de croissance durant la phase en eau douce. Il y a cependant eu une augmentation de l'hématocrite et une réduction significative de la concentration moyenne de chlorures dans le plasma ($p < 0,05$) chez le groupe moyen par comparaison au groupe témoin. Notre étude indique que les concentrations d'H^+ dans la gamme de pH 5,4–5,9 dans de l'eau qui contient peu ou pas d'Al réagissant avec la branchie n'affectent ni la croissance, ni la physiologie des saumons durant leur transformation en saumoneaux. Cependant, la réduction de l'hématocrite sanguin chez le groupe à pH bas trois mois après le transfert en eau de mer peut indiquer l'existence d'effets à long terme d'un tel traitement expérimental.

[Traduit par la Rédaction]

Introduction

There is extensive information on the biological effects of acidification of softwater streams in eastern North America and Scandinavia (Rosseland and Staurnes 1994; Gensemer and Playle 1999). Fish kills occur when water pH is low, and the pH reduction is often accompanied by an increase in Al. Aluminum toxicity is especially high when water pH is in the range 4.6–5.5 (Muniz and Leivestad 1980) but can be equally or more toxic at higher pH if present in unstable

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forms (Rosseland and Staurnes 1994). While negative effects related to Al are documented from Norwegian rivers having pH lower than 6, little to no effect is reported from Canadian rivers having pH in the range 5.0–5.5 (Lacroix 1989). The causes for this difference have received little attention, but a hypothesis is that major differences in organic content between Norwegian clearwater rivers and the organic rivers of Nova Scotia affect the determination of toxic concentrations of inorganic monomeric Al. The concentration of total Al is estimated to be higher in organic rivers, but inorganic Al is lower because organic matter ties up Al making it less available. By eliminating Al-related toxicity by complexing Al with citrate, we aimed to determine pH thresholds for Atlantic salmon (*Salmo salar*) smolts.

In the fish farming industry, the inlet water pH can be regulated to a set value of, for example, pH 6.5 by adding limestone, seawater, or silicate (Rosseland and Skogheim 1986; Jenssen and Leivestad 1989; Åtland et al. 1997). However, as respiration will increase the carbon dioxide concentration in the fish tanks, pH may drop to moderate acid conditions (Colt and Orwicz 1991; Fivelstad et al. 2003). The water carbon dioxide concentration in the effluent from Norwegian smolt farms is now often in the range 10–25 $mg \cdot L^{-1}$ and the corresponding pH between 5.5 and 6.5 (Maroni and Rosten 1998; Bergheim 1999; Fivelstad 1999). Even though Atlantic salmon may be exposed to moderately low pH both in streams and in smolt farms, there are few data on the long-term sublethal effects of the water H^+ concentration in isolation because most experiments are aimed towards the effects of Al (Magee et al. 2003). Furthermore, most studies on the physiological effects of low pH have been performed in the range 4.0–4.5. In this pH range, the physiological effect of pure acid exposure is failure in ion regulation at the gill surface, while respiratory failure is more predominant at pH levels higher than 5.5 and in the presence of Al (Neville 1985; Wood et al. 1988). The present experiment was carried out to study long-term sublethal effects of moderately low pH without elevated Al on Atlantic salmon smolts in freshwater. The response variables in the present investigation included ion regulatory, haematological, histomorphological, and growth parameters, measured during both the freshwater and marine phases.

Materials and methods

Experimental setup

The tap water used in the experiment came from Lake Svartediket, the water reservoir of Bergen City (western Norway). The water is soft (conductivity 24–32 $\mu S \cdot cm^{-1}$, Ca 0.4–0.9 $mg \cdot L^{-1}$, Cl 6–17 $mg \cdot L^{-1}$, Na 3–5 $mg \cdot L^{-1}$) and acidic (about pH 5) and contains total Al of ≈130 $\mu g \cdot L^{-1}$ (≈4.8 $\mu mol \cdot L^{-1}$). Description of the experimental tank facilities have been presented in earlier reports (Fivelstad and Binde 1994; Fivelstad et al. 1999). At the water inlet to the laboratory, trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$; ad purum from VWR International) was added at 14 $mg \cdot L^{-1}$ (48 $\mu mol \cdot L^{-1}$ or 10 times more citrate than Al) to complex Al. The Na concentration increased to 6–8 $mg \cdot L^{-1}$ (3 mg $Na \cdot L^{-1}$ was added) after the addition of trisodium citrate. The organic complex is not bioactive (Fivelstad and Leivestad 1984). Thereafter, the water was aerated in a packed column aerator in which excess nitrogen gas was removed and passed through a mechanical filter (Unik filter system, Os, Norway) to a header tank (450 L). In the header tank, pH was regulated to 7.2–7.3 by the addition of NaOH using an automatic pH-control system (Prominent Dulcotest PHD 1201).

A second Prominent Dulcotest PHD 1201 system was connected to an alarm transmitter (ROBOFON RBS-06) for low and high pH control. Sodium pyrosulfite was added using peristaltic pumps to remove chlorine. From the header tank, the water passed to rectangular mixing tanks. Diluted H_2SO_4 was added by peristaltic pumps (Alitea C6XV) to each mixing tank (20-min retention time) to produce the low and medium pHs. Pressurized air was applied to thoroughly mix the diluted H_2SO_4 in the water. The experimental tanks were covered and fitted with internal illumination. Each tank was illuminated by an incandescent bulb, which simulated a natural photoperiod of 60°N including twilight.

Fish stock, experimental design, and measurements of water quality

Aqua Gen-strain Atlantic salmon presmolts were obtained from Firda Fish Farm (western Norway) on 3 May 2000 and acclimated to the laboratory conditions for 2 weeks. The water at the Firda Fish Farm was relatively soft (conductivity 66 $\mu S \cdot cm^{-1}$, Ca 2.6 $mg \cdot L^{-1}$, total alkalinity 0.113 $mmol \cdot L^{-1}$, reactive Al 37 $\mu g \cdot L^{-1}$).

Six experimental tanks, representing duplicates of the three pH treatment groups, were each stocked with 85 smolts for 35 days of exposure in freshwater. The smolts were exposed to a simulated natural photoperiod of 60°N and were fed a commercial dry diet food (Ewos Micro 4 5A; Stavanger, Norway) in excess by automatic feeders every 15 s during light hours.

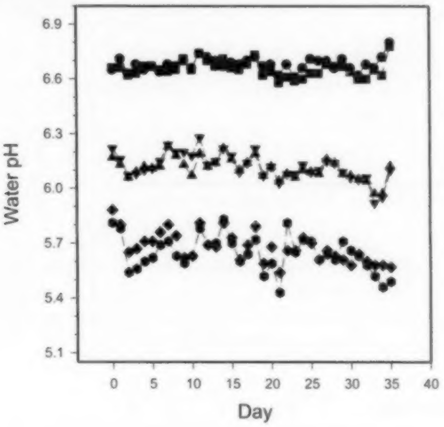
Freshwater pH was measured daily by a Radiometer PHM80 (PHC2701-7 pH electrode) in each tank (Fig. 1). Conductivity was 33–35 $\mu S \cdot cm^{-1}$, measured once a week using a YSI S-C-T meter (model 33). Water temperature, recorded daily, was between 7 and 11 °C. Dissolved oxygen, measured twice daily (YSI dissolved oxygen meters, model 57), was always above 8 $mg \cdot L^{-1}$. The water flow was 5 $L \cdot min^{-1}$ through all tanks at the start and 6 $L \cdot min^{-1}$ at the end of the experiment.

Citrate was added to complex and eliminate the toxic effects of Al. Water Al was analyzed and speciated only once during the experiment (day 27). Aluminum was immediately separated into two different fractions (reactive and nonlabile Al) by an Amberlite IR120 Na^+ exchanger. The two fractions were analyzed at Chemlab Services (Bergen, Norway) by the pyrocatechol method using an Ilachate Quik Chem 8000 (Zelweger Analytics, Milwaukee, Wis.). Labile Al (inorganic Al) includes mainly Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, and AlF^{2+} and is considered to be the toxic fraction of Al. This fraction was calculated as the difference between reactive and nonlabile Al (organic monomeric Al).

Seawater period

Fish from the two replicates of each treatment were combined into one tank at transfer to seawater. These postsmolts were then kept in 500-L square tanks, the water temperature was 9–10 °C, and the salinity was 34‰ (as measured by

Fig. 1. pH for the three groups during the freshwater period of Atlantic salmon smolts for the control group (●, ■ (replicates)), the medium-pH group (▲, ▼ (replicates)), and the low-pH group (◆, ●(replicates)).



WTW model LF191). The outlet oxygen content was maintained above 74%. The postsmolts were exposed to a simulated natural photoperiod and were fed a commercial dry diet (EWOS small (at the start) and medium (at the end)) by automatic feeders every eight minutes between 0800 and 1600.

Blood samples and analysis

Blood samples were taken from six smolts on day 0 and from six to eight smolts in each tank on days 7 and 32 during the freshwater period, 12 h after seawater transfer (day 36), and then 3 months after seawater transfer. From each tank, six to eight smolts were taken in a single catch and were rapidly transferred to an anaesthetic tray containing 0.8 mg metacaine$\cdot L^{-1}$, which produced almost immediate anaesthesia. After 2 min, the smolts were transferred to another tray containing 0.4 mg metacaine$\cdot L^{-1}$. Blood samples were drawn from each fish, within 15–40 s, by caudal venous puncture using 1-mL heparinized syringes. All blood samples were taken within 6–8 min in the anaesthetic solutions. The blood was centrifuged by a Sigma 201M for 5 min and haematocrit was measured. The plasma samples were immediately analyzed for Cl (Radiometer CMT 10 Chloride Titrator). Serum cortisol was analyzed with a ^{125}I radioimmunoassay kit (Gammacoat ^{125}I cortisol RIA; Baxter Healthcare Corporation, Deerfield, Ill.).

Immediately after blood sampling, the second gill arch on the right side of each fish was removed, cleaned on dry paper, immersed in SEI buffer (Zaugg and McLain 1982), and quickly frozen at –80 °C. Gill Na^+,K^+-ATPase activity was determined according to the method of McCormick (1993). Briefly, this kinetic assay utilizes the hydrolysis of ATP, which is enzymatically coupled to the conversion of NADH to NAD^+ by pyruvate kinase and lactic dehydrogenase with or without the addition of ouabain, a specific inhibitor of Na^+,K^+-ATPase. Readings were done at 340 nm for 10 min. Protein in homogenate was determined by a bicinchoninic acid method. Proteins react with Cu^{2+} and reduce it to Cu^+. The bicinchoninic acid reacts with Cu^+ and the resulting colour has a peak in absorbance at 562 nm. The results are expressed as micromoles ADP per milligram protein per hour.

Gill Al concentration was determined on the third gill arch from the left side of each fish. The gill filament was dissected and directly transferred to preweighed, acid-washed 20mL polyethylene plastic vials. The gills were stored frozen before being freeze-dried, weighed, and digested in HNO_3 (14 mol$\cdot L^{-1}$). Digested gill arches were diluted to 10% HNO_3 by Milli-Q water and the concentration of Al was determined using inductively coupled plasma – atomic emission spectroscopy (Kroglund and Finstad 2003). The results are presented as micrograms Al per gram dry weight gill.

Weight, length, condition factor, and histomorphological examination

Individual body weight and length were measured on all fish 6 days prior to exposure start (day –6), after 35 days of exposure in freshwater, and after 3 months in seawater. Condition factor (K) was calculated as

$$(1) \qquad K = 100wL^{-3}$$

where w is the wet weight (grams) of each fish and L is fork length (centimetres). Specific growth rate was calculated from the equation

$$(2) \qquad G_o = ((\ln W_2 - \ln W_1)/(t_2 - t_1)) \times 100$$

where G_o is the mean growth rate achieved (percent per day) and W_1 and W_2 are mean fish weights at sample times t_1 and t_2, respectively.

At days 0 (six fish) and 32 (12 fish from each group) smolts were pithed by cutting of the spinal cord just behind the opercula. Immediately after pithing, the second gill arch on the left side of the fish was fixed in 10% neutral buffered formalin. The gills were embedded in paraffin wax, and then sections were cut at 3–5 mm thickness and stained with haematoxylin and eosin (H & E). The slides were examined blind, i.e., without knowledge of classification with regards to exposure group.

Statistical treatment

Statistical analysis of the data was conducted using the software Statgraphics from STSC Inc. (Rockville, MD 20852). Multiple comparisons among pairs of means were performed by one-way analysis of variance combined with the Tukey test method (Sokal and Rohlf 1981; Fowler and Cohen 1990). The Kruskal–Wallis test was used for comparisons when significant differences were found among the standard deviations for a parameter. The χ^2 test was used to compare prevalence of gill lesions.

Results

The exposure pH conditions were maintained at a fairly stable level during whole exposure period (Fig. 1). pH was very similar in the replicates of each group. In the control group, pH was maintained at levels around 6.6. In the medium-pH group, pH was maintained at a level between 6.0 and 6.3, although pH dropped towards 5.9 the last exposure week. In the low-pH group, pH varied between 5.4 and

Table 1. Aluminum species ($\mu g \cdot L^{-1}$) (single measurements) in the raw water and in the tanks after addition of trisodium citrate.

		Control group (pH ≈ 6.7)		Medium-pH group (pH ≈ 6.1)		Low-pH group (pH ≈ 5.6)	
Al species	Raw water	Tank 1	Tank 6	Tank 2	Tank 4	Tank 3	Tank 5
Total Al	131	127	129	130	130	131	131
Reactive Al	41	<5	<5	6	6	9	8
Nonlabile Al	30	<5	<5	<5	<5	7	6
Labile Al	11	<5	<5	<6	<6	2	2

Note: Citrate was not added to the raw water. Labile Al (considered to be the toxic fraction) is the difference between reactive Al and nonlabile Al.

5.9; pH was also slightly lower in this group during the last exposure week.

The total Al concentration (about 130 $\mu g \cdot L^{-1}$, 4.8 $\mu mol \cdot L^{-1}$) was identical in all treatments. In the untreated source water, 41 $\mu g \cdot L^{-1}$ was present as reactive Al and 11 $\mu g \cdot L^{-1}$ as labile Al (Table 1). After addition of 14 mg trisodium citrate$\cdot L^{-1}$ (48 $\mu mol \cdot L^{-1}$), the reactive Al concentration was reduced to concentrations lower than 10 $\mu g \cdot L^{-1}$ and the concentration of labile Al was below 6 $\mu g \cdot L^{-1}$ in all exposure tanks.

The mean gill Al accumulation was significantly higher in the medium-pH group compared with the control group after 32 days ($p < 0.05$) (Fig. 2*a*). After 100 days in seawater, the mean gill Al concentration was about 2 µg Al·g dry weight gill^{-1} in all groups. The histomorphological examination revealed no significant lesions in gill tissue in any groups at day 32 of the freshwater period.

In general, no significant effects were found in blood chemistry parameters between the medium-pH group and the control group (Table 2; Figs. 2*b* and 2*c*), with exception of slightly but significantly lower plasma protein in the medium-pH group after 7 days ($p < 0.05$) (Table 2). In the low-pH group, mean blood haematocrit was significantly elevated after 32 days in freshwater, and after 100 days in seawater, haematocrit was significantly reduced when compared with the control group (Fig. 2*b*). No significant differences between the low-pH group and the control group were found in plasma cortisol levels and plasma protein levels during the freshwater and the seawater period (Table 2). Mean plasma Cl concentrations of the low-pH group were significantly reduced after 32 days of exposure ($p < 0.05$) (Fig. 2*c*). However, 12 h after seawater transfer, no significant differences between groups were observed in plasma Cl, and the mean plasma Cl levels were normal for all groups after 100 days in seawater. Mean gill Na$^+$,K$^+$-ATPase activity increased in all groups during the freshwater period. However, after 32 days in freshwater, mean gill Na$^+$,K$^+$-ATPase activity in the low-pH group was lower than in controls, although not significantly (Table 2). Plasma cortisol was much lower in seawater fish compared with freshwater fish (Table 2).

No mortality was observed during the freshwater period. During the last 2 weeks in seawater, the mortality was 0.7% (one postsmolt), 1.4% (two postsmolts), and 1.4% in the control group, medium-pH group, and low-pH group, respectively. There was no further mortality in any of the groups during the seawater period. There were no significant differences in mean weight, length, and condition factor among the groups during the freshwater period (Table 3). At the end of the seawater period, mean weight and mean condition factor for the low-pH group (during the freshwater period) were significantly higher than for the control group ($p < 0.05$).

Discussion

The fish were exposed to a water quality that mainly differed with respect to the H$^+$ concentration. The source water before treatment was acid and it contained Al. Since citrate was added to eliminate the toxic effects of Al, water Al was only analyzed once during the experiment. The measured concentrations of labile Al were lower than 6 $\mu g \cdot L^{-1}$. Despite the measured concentration being low, it cannot be concluded from this that Al had no biological effect. The uncertainty with respect to effect is in part due to the unstable properties of Al together with the analytical uncertainty present at these low levels of labile Al. The gill Al concentration may in these cases be a more sensitive indicator than the chemical analysis, where accumulation of Al onto the gill structure can be regarded as a response to exposure to bioavailable Al (Kroglund et al. 2001*a*, 2001*b*). Fish exposed under control and low-pH conditions lost gill Al during the freshwater phase compared with the initial level of 17 µg·g dry weight^{-1}, while fish exposed to medium pH accumulated Al to a concentration of 27 µg·g dry weight^{-1}. Kroglund and Finstad (2003) found marine survival to be reduced in wild salmon having gill Al concentrations in the range of 30–40 µg·g dry weight^{-1} and the medium-pH group is close to these levels. However, it should be noted that there were no significant physiological differences between the medium-pH and the control group during the experiment on day 32, and the water qualities did not have any effect on gill tissue structure. H$^+$ can therefore be identified as the main difference in water chemistry separating the different groups. Differences in gill Al between the low-pH group and the medium-pH group can be due to differences in time to reach some form for equilibrium and to differences in pH-related solubility (Lydersen et al. 1991).

Biological stress reactions can be released by numerous factors in aquaculture, including water quality parameters (Pickering 1981; Barton 1997). Salmonids exposed to acute stressors generally show an initial increase in plasma cortisol levels, after which the levels decline to normal levels, even if the stress is chronic (Schreck 1981; Sumpter 1997). Under severe chronic stress, however, plasma cortisol levels may remain above basal concentrations for weeks

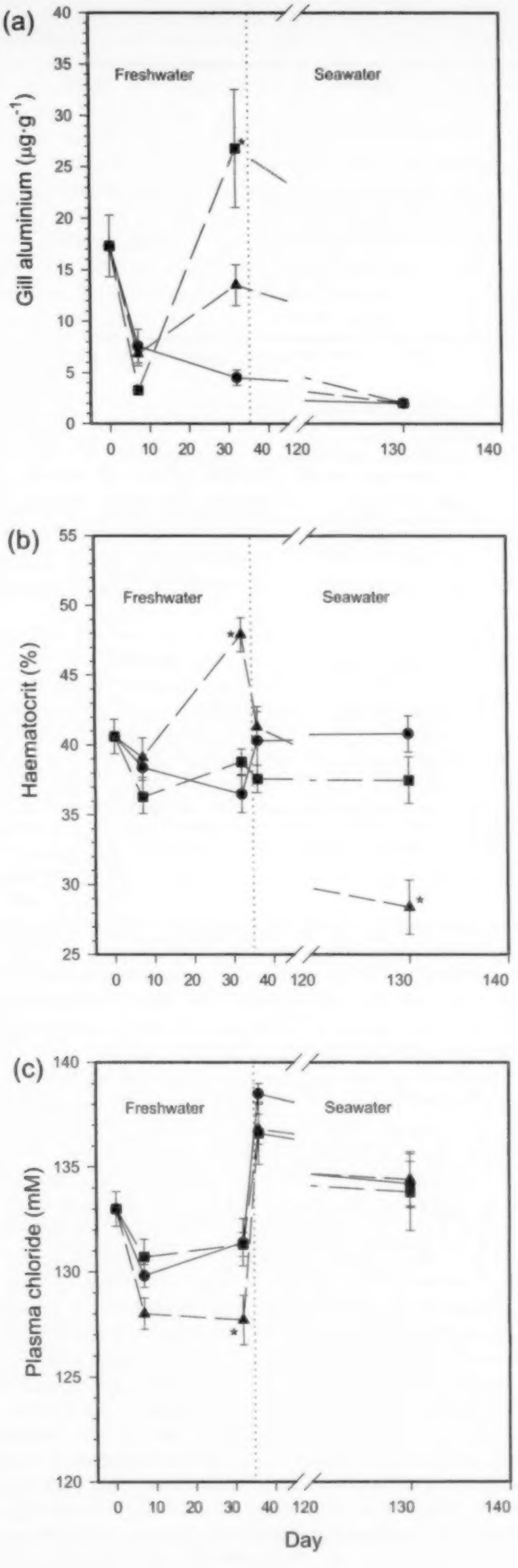


Fig. 2. Accumulation of Al in the gills and physiological parameters (during both the freshwater and seawater periods) of Atlantic salmon smolts for the control group (●), medium-pH group (■), and low-pH group (▲). Means and standard errors are given. Significant difference from control is denoted by an asterisk for $p < 0.05$ ($n = 6$ on day 0, $n = 12$ for each point on days 7 and 32 and for the first measurements in seawater (day 36), and $n = 6$ for each point at the end of the seawater period except for the low-pH group for which $n = 9$ for haematocrit and plasma Cl). (*a*) Gill (dry weight) Al concentration; (*b*) haematocrit; (*c*) plasma Cl.

(Pickering et al. 1991; Fou and Lam 1993; Barton 1997). During the present experiment, no significant differences in plasma cortisol levels were found between groups, suggesting that pH in the range 5.4–5.9 did not activate stress reactions. The decline in plasma cortisol observed during the experiment after transfer to seawater is unexpected and difficult to explain, based on the increase normally observed in smolts prior to seawater entry (Shrimpton et al. 1994) as well as the role of cortisol in maintaining hydro mineral balance in seawater (McCormick 2001). There are, however, few studies of long-term cortisol levels of salmon postsmolts in seawater; hence, the levels observed in the present study may represent normal resting cortisol levels in seawater-adapted Atlantic salmon postsmolts. It should also be noted that Knoph and Olsen (1994) found plasma cortisol levels in a control group of Atlantic salmon postsmolt to be in the range 0.0–0.4 µg·100 mL^{-1}.

Smoltification is a sensitive period in the salmon life cycle. During this period, several physiological processes change to adapt the smolt to survive a hyperosmotic environment. Among others, gill Na^+,K^+-ATPase activity increases to peak values in May–June (Stefansson et al. 1998). This is in agreement with the observed increase in gill Na^+,K^+-ATPase activity from day 0 to day 32 in the present investigation. For smolts in all groups, the Na^+,K^+-ATPase activity was in the normal range (Handeland et al. 1998). Thus, in the present experiment, the completion of the smoltification process did not seem to be affected at pH levels around 5.4–5.9, although activity was slightly reduced in the low-pH group. Staurnes et al. (1993) recorded a reduction in Na^+,K^+-ATPase activity in smolts exposed to pH 5.0 as compared with control pH levels. Exposure to Al enhanced the activity reduction. There might therefore be a toxicity threshold somewhere between pH 5.4 and 5.0.

The present investigation was performed in soft water containing low levels of Ca and moderate levels of Na and Cl. The toxicity of most toxicants is increased when the concentrations of Ca are low (Alabaster and Lloyd 1982). In soft water, Audet et al. (1988) observed transient net losses of Na and Cl during long-term acid exposure (pH 4.8) in rainbow trout (*Oncorhynchus mykiss*), which is in agreement with reduced plasma Cl values observed in the low-pH group in the present investigation.

Increased plasma protein has been observed in studies on acute acid exposure, probably resulting from changes in total plasma volume secondary to ionic disturbances (Milligan and Wood 1982). No significant differences in plasma protein levels were observed between groups, which indicates

Table 2. Atlantic salmon blood chemistry parameters (mean ± 1 SE) during the freshwater period (days 7 and 32) and seawater period (day 130).

Parameter	Day	Control group (pH 6.5–6.8)	Medium-pH group (pH 5.9–6.3)	Low-pH group (pH 5.4–5.9)
Plasma cortisol (μg·100 mL^{-1})	7	5.5±1.20 (12)	2.4±0.34 (12)	4.2±0.50 (12)
	32	5.9±1.20 (12)	5.4±1.37 (12)	4.1±0.51 (12)
	130	0.6±0.14 (6)	0.7±0.08 (6)	0.5±0.09 (9)
Plasma protein (g·L^{-1})	7	38.9±1.35 (12)	35.0±1.09 (12)*	36.8±1.46 (12)
	32	33.8±0.96 (12)	34.8±0.62 (12)	37.7±0.94 (12)
	130	36.3±3.3 (6)	31.1±3.9 (6)	33.1±2.7 (6)
Na$^+$,K$^+$-ATPase (μmol ADP·mg protein^{-1}·h^{-1})	0	8.6±0.79 (6)	8.6±0.79 (6)	8.6±0.79 (6)
	32	19.9±1.36 (12)	22.6±1.05 (12)	17.0±0.51 (12)

Note: Numbers of samples are given in parentheses. Significant difference from control is noted by an asterisk for $p < 0.05$.

Table 3. Atlantic salmon weight, specific growth rate, length, and condition factor (mean ± 1 SE) during the freshwater period (days 0–35) and seawater period (days 36–133).

Parameter	Day	Control group (pH 6.5–6.8)	Medium-pH group (pH 5.9–6.3)	Low-pH group (pH 5.4–5.9)
Weight (g)	–6	42.3±0.52 (120)	42.3±0.52 (120)	42.3±0.52 (120)
	34	63.7±0.74 (138)	61.4±0.82 (137)	62.9±0.94 (114)
	133	181.8±4.41 (98)	181.7±4.74 (104)	202.8±6.85 (81)*
Specific growth rate (%·day^{-1})	–6–34	1.02	0.93	0.99
	34–133	1.06	1.10	1.18
Length (cm)	–6	15.6±0.07 (120)	15.6±0.07 (120)	15.6±0.07 (120)
	34	18.1±0.09 (138)	17.7±0.10 (137)*	17.9±0.11 (114)
	133	25.1±0.27 (98)	24.8±0.29 (104)	25.5±0.37 (81)
Condition factor	–6	1.11±0.01 (120)	1.11±0.01 (120)	1.11±0.01 (120)
	34	1.08±0.007 (138)	1.10±0.008 (137)	1.10±0.011 (114)
	133	1.13±0.009 (98)	1.16±0.011 (104)	1.19±0.015 (81)*

Note: Numbers of samples are given in parentheses. Significant difference from control is noted by an asterisk for $p < 0.05$. It should be noted that the three groups were exposed to similar conditions in seawater (days 36–133). Day –6 means 6 days before the start of the experiment.

that the plasma volume in the smolts was not changed significantly by the present pH-rearing regimes. The significantly increased haematocrit level of the low-pH group during freshwater exposure is a typical response following exposure to acidified water and can be due to both erythrocyte swelling and loss of plasma water (Witters et al. 1987). Increased haematocrit can indicate acute stress, while low values indicate anemia resulting from chronic stress (Iwama et al. 1997). The reason for the large reduction in blood haematocrit in the low-pH group 3 months after seawater transfer is not clear but it does imply a long-term sublethal effect of the treatment. This group had at the same time the lowest plasma Cl concentration and lowest Na$^+$,K$^+$-ATPase activity prior to seawater entry.

Gjedrem (1980*a*) carried out a 4-month growth experiment to study comparative effects of water pH on growth rate in rainbow trout, Arctic char (*Salvelinus alpinus*), and brown trout (*Salmo trutta*). The three species had similar growth curves at pH 5.5 and pH 6.2. Although significant differences in acid tolerance among species of salmonids have been found (Gjedrem 1980*b*; Poléo et al. 1997), the result of the present experiment does not indicate that pH in the range 5.4–6.8 affects growth or mortality when Al toxicity is reduced or eliminated by complexation. In fact, the low-pH group was heavier and had higher condition factor after transfer to seawater. The reason for why these fish had the highest condition factor in seawater is unclear but it can suggest overcompensation.

Fivelstad et al. (2003) reported an association between gill Al accumulation and gill lesions, i.e., mainly chloride cell hyperplasia and adhesion of filaments in water containing Al in the pH interval 5.4–5.8. The absence of gill tissue changes in the present study suggests that labile Al was the causative factor of the gill lesions in the previous study. This is in accordance with Karlsson-Norrgren et al. (1986), Fischer-Scherl and Hoffman (1988), and Smith and Haines (1995), who all speculated that the pathological changes developed because of the effect of labile Al rather than low pH alone. The study of Fivelstad et al. (2003), performed in the same pH range as the present study, showed that an accumulation of water carbon dioxide, a reduction in water pH, and the changes in Al chemistry may be detrimental to Atlantic salmon smolts, even at low Al concentrations. Therefore, even though pH in isolation may not have toxic effects on the smolts, the combination low pH, high carbon dioxide, and moderate concentrations of toxic Al should be avoided.

The results also suggest a possible cause for the apparent differences in water quality requirements observed between organic-rich rivers in Nova Scotia and Norwegian clearwater rivers. Exposure to low pH alone does not impose any major sublethal stress having any detectable adverse effect on marine survival and growth. Despite high levels of labile Al in the Canadian rivers, gill Al was reported to be low, and lower than is to be expected based on data from Norwegian experiments performed on organic-poor water (Kroglund et al. 2001*a*, 2001*b*). Addition of citrate binds labile Al, thus mimicking higher levels of dissolved organic C. The results indicate that the observed differences in water quality between the regions can be related to concentration of bioavailable Al and that the H^+ concentration is not the cause of population declines before pH falls below 5.5. The actual accumulation of Al onto the gill structure can be a better predictor of Al-related toxicity than interpretations of water quality based on Al fractionations.

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Preservation of genetic diversity in restocking of the sea cucumber *Holothuria scabra* investigated by allozyme electrophoresis

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Abstract: Population genetics analyses should be considered when releasing hatchery-produced juveniles of the sea cucumber *Holothuria scabra* when spawners from nonlocal populations are used. In New Caledonia, within-region genetic heterogeneity of *H. scabra* populations (examined through allozyme electrophoresis of 258 animals) indicated high gene flow between nine sites and F_{ST} values did not deviate significantly from zero. However, exact tests indicated that populations at two sites with limited water exchange in the southern location were significantly different from populations at three other locations on the west coast. Inclusion of *H. scabra* sampled in Bali ($n = 90$) and Knocker Bay, Australia ($n = 47$), and comparisons with existing data from the west Pacific (Torres Strait, Solomon Islands, Upstart Bay, Hervey Bay) showed that populations were significantly different (using exact tests) and samples partitioned distinctly using unweighted pair group method with arithmetic mean clustering. Rogers' genetic distance values between populations were significantly related to geographic distances, showing a pattern of isolation by distance. The rapid increase in genetic distance over the first few hundred kilometres supports the view that the spatial extent of any translocation needs to be carefully considered on the basis of knowledge of variation in allele frequencies within the target area.

Résumé : Lorsqu'on ensemence en nature des jeunes *Holothuria scabra* élevés en pisciculture et issus de parents qui n'appartiennent pas aux populations locales, il est indiqué de penser faire des analyses génétiques de population. En Nouvelle-Calédonie, l'hétérogénéité génétique des populations d'*H. scabra* au sein d'un même région (basée sur l'examen par électrophorèse des allozyme de 258 individus) démontre l'existence d'un important flux génétique entre neuf sites; de plus, les valeurs de F_{ST} ne diffèrent pas significativement de zéro. Cependant, des tests précis montrent que les populations à deux sites du sud, qui font peu d'échange d'eau, diffèrent significativement de trois autres populations de la côte occidentale. L'inclusion de spécimens d'*H. scabra* prélevés à Bali ($n = 90$) et à la baie Knocker en Australie ($n = 47$), de même que la comparaison avec les données existantes pour le Pacificique ouest (détroit de Torres, îles Solomon, baie Upstart et baie Hervey), indiquent que les populations sont significativement distinctes (à l'aide de tests précis) et que les échantillons se séparent de façon nette dans une analyse de groupement de type UPGMA (méthode de groupement par associations moyennes UPGMA). Les valeurs de distance génétique de Rogers entre les populations sont en corrélation significative avec les distances géographique, ce qui indique un système d'isolement par la distance. L'accroissement rapide de la distance génétique sur les premières centaines de kilomètres appuie l'opinion que la répartition spatiale de toute translocation doive être prise en compte sérieusement à partir de ce qu'on connaît de la variation dans les fréquences d'allèles dans la région ciblée.

[Traduit par la Rédaction]

Introduction

About 20 species of sea cucumbers of the order Aspidochirotida are fished commercially in the tropical Indo-Pacific. The processed body wall, known as "bêche-de-mer" or "trepang", is exported to Asia, where it is consumed as a delicacy, an aphrodisiac, or a natural medicine. Fueled by an increase in trade with China, widespread overfishing of sea cucumbers in most countries over the past decades has depleted stocks of commercially important species (Conand 1997, 2001; Uthicke and Benzie 2000*a*).

The species of highest value, the sandfish *Holothuria scabra*, is overfished in many regions because it often occurs in easily accessible, shallow coastal waters (Conand 1989; Hamel et al. 2001). Although countries such as Australia, Fiji, and the Solomon Islands have imposed long-term fishing closures for this species, there is some evidence that natural recovery of sea cucumber stocks may take several

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decades (Battaglene and Bell 1999; Lincoln-Smith et al. 2000; Uthicke et al. 2004).

Following the development of culture methods for *H. scabra* (Battaglene 1999; James 1999; Pitt 2001), many countries are now considering restocking with hatchery-produced juveniles as a way to accelerate the recovery of sizeable breeding populations (Battaglene and Bell 1999). This strategy raises concerns about altering the genetic diversity of remnant stocks (see Hindar et al. 1991; Utter 1998) because it may be desirable to translocate the hatchery-produced juveniles to sites other than where the adult broodstock were collected.

Release of hatchery-produced animals can cause reduced genetic diversity in wild stocks in two ways. Firstly, if broodstock used for hatchery production are from different genetic stock, the native alleles may be reduced in frequency through introgression of exogenous alleles. This introgression may be caused either by interbreeding of animals from the two stocks or by the introduced alleles outcompeting native alleles because of higher fitness of individuals carrying these introduced alleles (Hindar et al. 1991; Utter 1998). Thus, introgressions may reduce genetic differences among stocks (Utter 1998). Secondly, interbreeding of introduced stock with the native stock may disturb potentially complex adaptations to the local environment that have evolved over time, causing "outbreeding depression" (Templeton 1986; Waples 1995). Such hybridizations tend to have long-lasting effects that are disadvantageous to native stocks rather than beneficial through added genetic variation (Hindar et al. 1991). Therefore, unless the genetic structures of stocks at release and source sites are known, juveniles should be released only at native sites to preserve the genetic diversity of stocks (Shaklee and Bentzen 1998). These cautions arise mostly from translocations of temperate fishes but could be more pertinent for sedentary invertebrates with limited postsettlement dispersal such as *H. scabra*, which moves at most 1–2 m·day^{-1} (Hamel et al. 2001) and probably only a couple of kilometres in a lifetime.

However, even when native populations are used, the number of spawners must be carefully chosen to avoid a reduction in overall effective population sizes (Ryman et al. 1995), especially in highly fecund species with large variations in reproductive success (Tringali and Bert 1998), such as holothurians. A reduction in effective population size can lead to losses of genetic variation, for example through decreased heterozygosity or the loss of rare alleles (Utter 1998). Another potential problem when releasing animals bred in captivity into the environment is domestication selection, which can lead to a high supplementation load of deleterious alleles in wild populations (Lynch and O'Hely 2001). Hardly any work on these issues has been conducted for marine invertebrates. However, studies on abalone (*Haliotis* spp.) have provided some indication for reduced diversity in hatchery-produced animals (Mgaya et al. 1995; Gaffney et al. 1996).

In general, long-lived marine invertebrate larvae provide an efficient means of gene flow between populations, leading to small population differentiation even over large geographic scales (Avise 1994; Palumbi 1997). Within the Pacific, *Holothuria nobilis* (Uthicke and Benzie 2000*b*, 2003) and a variety of other echinoderms show very little population differentiation over distances larger than 1000 km (e.g., Williams and Benzie 1998; Benzie 1999; McCartney et al. 2000).

Holothuria scabra is a broadcast spawner with a relatively long planktotrophic larval duration of 10–14 days in hatchery culture (Battaglene et al. 1999; Mercier et al. 1999), although larvae could survive longer in the wild in low concentrations of algae. Unlike the examples above, allozyme genetic studies on *H. scabra* (Uthicke and Benzie 1999, 2001) indicated that gene flow between populations greater than ~800 km apart was more restricted than expected from estimates of larval duration. In one instance, significant genetic differentiation occurred between sites separated by only 16 km, possibly because of the geomorphology at one site that reduced larval connectivity and severe depletion of one stock through overfishing (Uthicke and Benzie 2001).

This study used allozyme electrophoresis to determine genetic differentiation of *H. scabra* among multiple sites along New Caledonia, which are under consideration as release sites of cultured *H. scabra* in a project designed to determine optimal restocking methods (Purcell et al. 2002). We use these data to examine patterns of within-region genetic heterogeneity of *H. scabra* with a view to determining whether hatchery-produced juveniles from spawners collected at a single site could be released at other sites 60–400 km away. In addition, we investigated larval connectivity on a larger geographic scale by comparing data obtained in New Caledonia with previously published data from other Pacific regions and two additional populations in the Indo-Pacific. With this approach, we aim to assist restocking programs through an improved understanding of the genetic differentiation between stocks of *H. scabra* at different spatial scales so that the release of cultured juveniles can be planned in a responsible way to preserve the genetic diversity of existing stocks.

Materials and methods

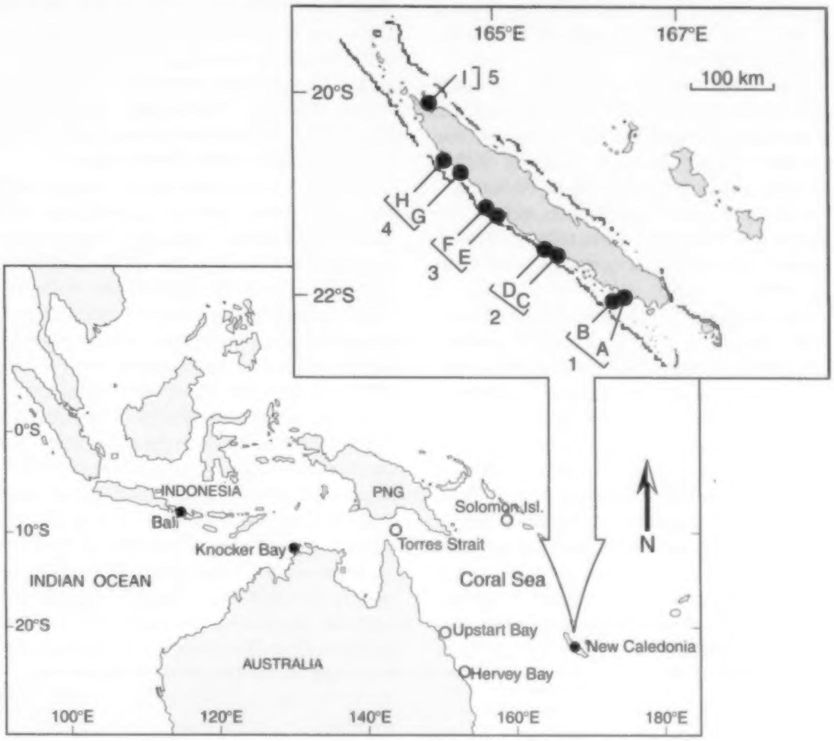
Study region

Sea cucumbers are diverse and ubiquitous in New Caledonia (Conand 1989). The expansive shallow habitats of the lagoon around the main island (La Grande Terre, hereafter referred to as New Caledonia) covering approximately 24 000 km^2 are enclosed by the second largest barrier reef in the world (Fig. 1). Mangrove systems and shallow fringing coral reefs with sandy or muddy sea grass beds are abundant along the west coast of the island. A number of the inshore holothurian species have been fished periodically since the 1840s, especially the sandfish *H. scabra* (Conand 1989). This species is common mostly on inshore fringing reef flats and bays <5 km from the mangrove systems, but there is evidence of stock depletion at some sites because of intense fishing.

Sampling

Five locations, each separated by 60–130 km, were selected along the coast of New Caledonia (Fig. 1). Two replicate sites with *H. scabra*, 8–23 km apart, were then chosen within four of these locations on the west coast of the island, whereas only one site was selected at the single northeast lo-

Fig. 1. Sampling sites (solid circles) for *H. scabra* in New Caledonia, Knocker Bay (Australia), and Bali (Indonesia). The barrier reef around New Caledonia is indicated with thick lines, letters denote sampling sites, and numbers denote locations. Sites: A, Baie de Sainte-Marie; B, Ilot Maître; C, Ouano; D, Ilots Kundogi; E, Plateau de Béco; F, Pointe Pindaï; G, Pouangué; H, Ouaco; I, Récif Thaavaam (Ilot Cocotier). Locations of previous samples from other regions (Uthicke and Benzie 2001) are indicated with open circles.



cation. Adult *H. scabra* (n = 14–34) were collected by hand from the nine sites (labelled A to I; see Fig. 1) in the five locations within search areas of <2 ha of sandy or muddy sea grass beds, generally on the shoreward sections of reef flats.

The viscera of each animal were removed from the body cavity through a 5- to 8-cm incision in the ventral body wall. A short section (5–10 cm) of the upper intestine without sediments was dissected, placed in a labelled plastic bag, and snap-frozen in liquid nitrogen. The tissue samples were stored in liquid nitrogen for 2 weeks during transport to the laboratory at the Australian Institute of Marine Science, Townsville, Australia.

In addition to samples from New Caledonia, we analysed data from samples obtained from populations at Knocker Bay, Northern Territory, Australia (11°19.4′S, 132°06.9′E), in May 2001) and Bali (8°06.0′S, 114°43.2′E) in March 1999 (S. Uthicke and J.A.H. Benzie, unpublished data). A cluster analysis and calculations of genetic distances were also conducted using data from *H. scabra* populations in northern Australia (Hervey Bay, Upstart Bay, Torres Strait) and in the Solomon Islands, published in Uthicke and Benzie (2001).

Electrophoresis

Electrophoresis of seven polymorphic enzyme loci was conducted according to Uthicke and Benzie (1999, 2001). In short, 180–250 mg of frozen gut tissue was homogenized in 250 μL of Tris–HCl buffer (100 mmol·L^{-1} Tris adjusted to pH 8.0 with HCl). Electrophoresis was performed on 12% horizontal starch gels using two different buffer systems. TEC7.9 gels (electrode buffer: 135 mmol·L^{-1} Tris, 32 mmol·L^{-1} citric acid, 4 mmol·L^{-1} Na$_2$EDTA, pH 7.9; gel buffer: 8.5 mmol·L^{-1} Tris, 2 mmol·L^{-1} citric acid, 0.27 mmol·L^{-1} Na$_2$EDTA, pH 7.9) were used to screen for variations in glucose-6-phosphate isomerase (EC 5.3.1.9, *GPI**), hexokinase (EC 2.7.1.1, *HK**), and phosphoglucomutase (EC 5.4.2.2, *PGM**). TG8.4 gels (electrode and gel buffer: 25 mmol·L^{-1} Tris, 192 mmol·L^{-1} glycine, pH 8.4) were used to screen for variations in malate dehydrogenase (EC 1.1.1.37, *MDH**) and peptidases (EC 3.4.11/13). The peptidase loci were reported as peptidase locus 1 (*PEP-1**), *PEP-2**, and *PEP-3** (Uthicke and Benzie 2001).

Statistical analyses

Basic analyses of genetic variability (allele frequencies, number of polymorphic loci, heterozygosity) were carried out using programs in the BIOSYS package (Swofford and Selander 1981). The allelic richness for each population was calculated following El Mousadik and Petit (1996) using FSTAT (Goudet 2001). The F statistics, genetic distance calculations, unweighted pair group method with arithmetic mean (UPGMA) cluster analyses (using 1000 permutations for bootstrapping), and Mantel tests were performed using the TFPGA (tools for population genetic analyses) package

(Miller 1997). Weir and Cockerham's (1984) methods for calculating Wright's F statistics with corrections for unequal sample size were used, partitioning total genetic variation (F_{IT}) into that occurring within populations (F_{IS}) and that occurring between populations (F_{ST}). The 95% confidence intervals of the average values were calculated by bootstrapping across loci with 5000 replicates.

Deviations from Hardy–Weinberg equilibrium for each locus–location combination were tested by exact test on pooled genotypes as implemented in TFPGA. Exact tests for population differentiation (Raymond and Rousset 1995) were conducted using 1000 dememorization steps, 10 batches, and 2000 permutations per batch. Significance values for Hardy–Weinberg equilibrium and exact tests were corrected for multiple simultaneous tests with a sequential Bonferroni correction (Hochberg 1988).

To test for isolation by distance as the cause of population structure, we plotted the logarithm of Rogers' (1972) genetic distance against the logarithm of the geographic distance between populations (in kilometres, derived from a geographic information system). As suggested by Slatkin (1993), a standard regression line was fitted. Outliers from this regression line were defined as data points with standard residuals >2.0. Because data points were not independent, a Mantel (1967) test was used to test for matrix correlation. These tests used genetic distances between all populations thus far analysed, with one exception: the population from Solomon Islands site A was omitted because it represented a sample from an isolated lagoon (Uthicke and Benzie 2001). The significance of Mantel's normalized Z was tested by 999 random permutations using TFPGA.

Results

Most allozyme loci investigated were highly variable and two to five alleles could be reliably scored (Table 1). The only exception was *MDH**, which displayed fixed or near-fixed alleles in each population, similar to previous studies on *H. scabra* (Uthicke and Benzie 2001).

Genetic variability within sites (e.g., allelic richness, number of polymorphic loci, heterozygosity) did not show any distinct differences between populations sampled in New Caledonia, Knocker Bay, and Bali (Table 2). The observed heterozygosity is, in each case, very close to that expected under Hardy–Weinberg equilibrium. Exact tests for deviations from Hardy–Weinberg equilibrium for each locus at each site indicated no significant deviations ($P > 0.05$) after corrections for multiple tests, indicating that all *H. scabra* populations investigated in New Caledonia, Knocker Bay, and Bali were in Hardy–Weinberg equilibrium. Genotypic diversity (expressed as number of genotypes observed, N_{go}) was high in each population, but some genotypes occurred more than once (Table 2).

Exact tests for population differentiation indicated no significant difference ($P > 0.05$) between any sites within locations along the coast of New Caledonia. Therefore, data from sites within locations were pooled for all subsequent analyses to increase statistical power of the analyses. The F_{ST} statistics for data from New Caledonia (Table 3) show low F_{ST} values for each locus, and the confidence intervals of the averages include zero. Therefore, this analysis did not indicate restrictions in gene flow within New Caledonia. Rogers' (1972) genetic distance was low between all pairs of locations (Table 4). However, the distances between location 1 and locations 2, 3 and 4 were higher than other comparisons. This was confirmed by exact tests of population differentiation between locations: after Bonferroni corrections, the pooled population from location 1 was significantly different from those at locations 2, 3, and 4. No significant difference existed for other between-location comparisons in New Caledonia.

Within New Caledonia, a cluster analysis illustrated the larger difference between populations at location 1 and those at other locations (Fig. 2). Populations from each of the large geographic regions (New Caledonia, Hervey Bay, Upstart Bay, Torres Strait, Solomon Islands, Northern Territory, Bali) form separate clusters, which are in most cases supported by high bootstrap values obtained from 1000 permutations. The two populations west of the Torres Strait (i.e., Bali and Knocker Bay) show the largest genetic differences from the other populations. Differences between all populations from the main geographic regions were highly significant (exact tests, all P values <0.002). The analysis also suggested that the populations most closely related to those in New Caledonia are from Hervey Bay (south Queensland).

Over the geographic distances covered in the present and in a previous study (Uthicke and Benzie 2001) (approximately 20–6000 km), Rogers' genetic distance between populations distinctly increased as a function of geographic distance (Fig. 3). Seventy-one percent of the variation in the (log-transformed) genetic distance values was explained by a linear regression ($y = 0.0324x - 0.047$) using (log-transformed) geographic distance as an independent variable. A Mantel (1967) test (normalized Z: 0.84, $P = 0.001$) confirmed that geographic and genetic distances were highly correlated, thus supporting the pattern of isolation by distance. However, this analysis also identified some distinct outliers to this pattern (Fig. 3).

Discussion

Allozyme genetic studies conducted to assist in the choice of sites for restocking experiments and future restocking indicated generally high gene flow between populations of *H. scabra* at New Caledonia, although the southernmost population was significantly differentiated from all others. In general, genetic variability (e.g., expected heterozygosity: 0.24–0.29) in *H. scabra* within sites was high along New Caledonia and at a level similar to that of populations at sites elsewhere in the region studied previously by Uthicke and Benzie (2001). Also similar to that study, we found the variability in fully tropical populations (Bali and Knocker Bay, Australia) to be somewhat elevated, which may provide some indication of a tropical origin of this species.

All populations of *H. scabra* studied were in Hardy–Weinberg equilibrium and there were no indications that asexual reproduction is part of the biology of this species, as was previously described (Uthicke and Benzie 2001). In other holothurian species, which can reproduce asexually by transverse fission, most loci at all populations deviated from

Table 1. Allele frequencies at seven loci of *H. scabra* from 11 sites.

	New Caledonia										
Locus	A	B	C	D	E	F	G	H	I	Bali	KB
*PGM**	(93)	(14)	(28)	(31)	(30)	(28)	(33)	(28)	(30)	(87)	(35)
100	0.574	0.214	0.427	0.452	0.533	0.482	0.485	0.429	0.583	0.259	0.486
94	0.000	0.000	0.018	0.032	0.000	0.018	0.000	0.018	0.017	0.149	0.142
90	0.132	0.357	0.161	0.129	0.167	0.089	0.167	0.161	0.100	0.052	0.086
86	0.118	0.214	0.268	0.274	0.200	0.286	0.242	0.304	0.217	0.540	0.286
80	0.177	0.214	0.125	0.113	0.100	0.125	0.106	0.089	0.083	0.000	0.000
*HK**	(34)	(14)	(28)	(31)	(30)	(28)	(33)	(28)	(30)	(89)	(45)
100	0.794	0.821	0.696	0.710	0.817	0.714	0.849	0.786	0.817	0.388	0.556
94	0.206	0.179	0.304	0.290	0.183	0.286	0.152	0.214	0.183	0.601	0.444
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000
*GPI**	(34)	(14)	(29)	(31)	(30)	(28)	(33)	(28)	(30)	(91)	(46)
113	0.059	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.033	0.022	0.011
100	0.912	1.000	0.983	0.984	0.933	0.929	0.956	0.964	0.933	0.967	0.978
90	0.029	0.000	0.017	0.016	0.050	0.071	0.046	0.036	0.033	0.011	0.011
*MDH**	(34)	(14)	(29)	(31)	(30)	(28)	(33)	(28)	(30)	(91)	(44)
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.989
91	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
*PEP-1**	(34)	(14)	(27)	(30)	(30)	(28)	(32)	(28)	(30)	(91)	(41)
106	0.206	0.357	0.352	0.233	0.250	0.196	0.156	0.268	0.233	0.445	0.902
100	0.794	0.623	0.648	0.767	0.750	0.804	0.844	0.732	0.767	0.555	0.097
*PEP-2**	(34)	(14)	(28)	(31)	(30)	(28)	(33)	(28)	(30)	(91)	(38)
100	0.795	0.929	0.946	0.984	0.967	1.000	0.955	1.000	0.900	0.989	0.829
94	0.235	0.071	0.054	0.016	0.033	0.000	0.045	0.000	0.100	0.011	0.171
*PEP-3**	(34)	(14)	(28)	(31)	(30)	(28)	(33)	(28)	(30)	(88)	(39)
100	0.927	0.893	0.875	0.839	0.833	0.893	0.909	0.821	0.817	0.568	0.667
95	0.073	0.107	0.125	0.161	0.167	0.107	0.091	0.179	0.183	0.432	0.333

Note: Numbers in parentheses indicate the sample size at each locus. KB, samples from Knocker Bay, Australia. See Fig. 1 for names of sample sites within New Caledonia. *PGM**, phosphoglucomutase; *HK**, hexokinase; *GPI**, glucose-6-phosphate isomerase; *MDH**, malate dehydrogenase; *PEP-1**, *PEP-2**, and *PEP-3**, peptidase loci 1, 2, and 3.

Table 2. Summary statistics and parameters describing genetic variability at seven loci of *H. scabra* from 11 sites.

			Mean heterozygosity			
Sample site and location	Allelic richness	% of loci polymorphic	Observed (H_o)	Expected (H_e)	N_i	N_{go}
Site A	2.2 (0.4)	85.7	0.265 (0.068)	0.286 (0.073)	34	28
Site B	2.0 (0.4)	71.4	0.265 (0.097)	0.268 (0.104)	14	14
Pooled location 1	2.3 (0.4)	85.7	0.265 (0.073)	0.287 (0.084)	48	41
Site C	2.1 (0.5)	85.7	0.294 (0.111)	0.282 (0.100)	24	23
Site D	2.1 (0.5)	85.7	0.265 (0.099)	0.264 (0.098)	30	24
Pooled location 2	2.2 (0.5)	85.7	0.278 (0.103)	0.272 (00.098)	54	41
Site E	2.1 (0.4)	85.7	0.276 (0.100)	0.259 (0.083)	30	24
Site F	2.1 (0.5)	71.4	0.224 (0.093)	0.249 (0.092)	28	24
Pooled location 3	2.3 (0.5)	85.7	0.251 (0.096)	0.254 (0.086)	58	41
Site G	2.1 (0.4)	85.7	0.235 (0.098)	0.221 (0.084)	32	23
Site H	2.0 (0.5)	71.4	0.260 (0.101)	0.259 (0.097)	28	25
Pooled location 4	2.2 (0.5)	85.7	0.247 (0.098)	0.239 (0.089)	60	44
Site I (location 5)	2.3 (0.4)	85.7	0.271 (0.082)	0.274 (0.073)	30	25
Bali	2.2 (0.4)	85.7	0.286 (0.092)	0.312 (0.102)	82	61
Knocker Bay	2.3 (0.3)	100	0.288 (0.085)	0.307 (0.092)	28	25

Note: Standard errors are given in parentheses where appropriate. Calculations of N_i (number of individuals) and N_{go} (number of genotypes observed) are based only on individuals in which all loci could be scored.

Table 3. *F* statistics for comparisons of populations of *H. scabra* among the five locations in New Caledonia (see Fig. 1).

Locus	F_{IT}	F_{ST}	F_{IS}
*PGM**	–0.0262	0.0020	–0.0282
*HK**	0.0087	0.0042	0.0045
*GPI**	0.0452	0.0029	0.0425
*MDH**	nt	nt	nt
*PEP-1**	–0.0101	–0.0034	–0.0067
*PEP-2**	0.1405	0.0741	0.0717
*PEP-3**	0.1191	–0.0025	0.1213
Average	0.0167	0.0057	0.0111
Upper 95% CI	0.0878	0.0245	0.0741
Lower 95% CI	–0.0147	–0.0014	–0.0174

Note: Upper and lower 95% confidence intervals (CI) are derived from bootstrapping over loci with 10 000 replicates. nt, test not performed because locus is monomorphic.

Table 4. Rogers' (1972) genetic distances (above diagonal) and *P* values of the exact tests for population differentiation (below diagonal) in *H. scabra* populations from five locations within New Caledonia.

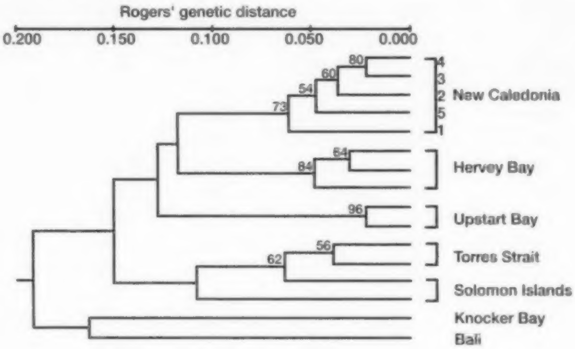
	1	2	3	4	5
1	—	0.0725	0.0607	0.0606	0.0527
2	**0.0002**	—	0.0365	0.0383	0.0620
3	**0.0014**	0.4565	—	0.0221	0.0394
4	**0.0003**	0.4482	0.9843	—	0.0417
5	0.1962	0.1073	0.3935	0.3877	—

Note: Bold type indicates significant (α = 0.05) population differentiation after sequential Bonferroni corrections for 10 multiple simultaneous tests.

Hardy–Weinberg equilibrium, and the genotypic diversity was significantly reduced (Uthicke et al. 1998, 1999, 2001).

The significant differences in allele frequencies between location 1 and most other locations along New Caledonia could be explained by the local geomorphology, which limits its water exchange with the more open lagoon system. Baie de Sainte-Marie (site A) is the only deep (20 m) enclosed bay among the study sites, so gene flow with other sites may be limited because of retention of *H. scabra* larvae in eddies (Douillet et al. 2001) and limited input from other populations. Ilot Maître (site B) is a national marine reserve and the only site surrounded by a near-continuous rim of reef crest. In contrast, the remaining seven sites were reef flats or shallow bays with unrestricted current flow to the lagoon. However, the genetic distance between location 1 and the other locations on the west coast of New Caledonia (average Rogers' (1972) distance: 0.065) is relatively small compared with the genetic distance found between the two populations in the Solomon Islands (0.120), where one site was within an enclosed lagoon. The surface current within the lagoon surrounding New Caledonia is predominantly driven by wind in a northwest direction, on average ~0.1 $m \cdot s^{-1}$ near the coast (Douillet et al. 2001). Therefore, mediated by this current, larvae could drift ~90 km during a 10-day larval period. However, the fact that populations in the south were significantly different from other populations suggests that gene flow between populations is restricted to some extent. Thus, our study supports the findings by Uthicke and Benzie (2001) that significant genetic differences can exist in *H. scabra* populations over relatively short distances. It is also consistent with their finding that restricted gene flow can occur between sites with entraining hydrographic conditions that would enhance self-recruitment of local larvae to native sites and limit dispersal to sites in open systems.

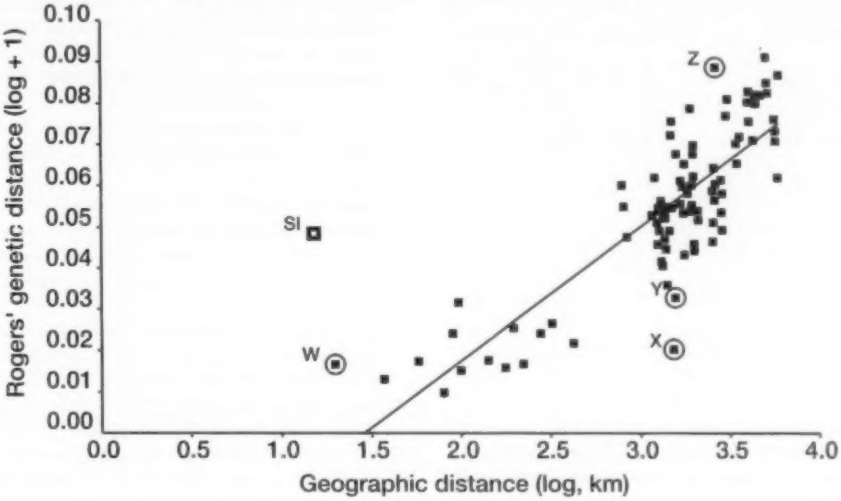
Fig. 2. Dendrogram illustrating the genetic relationships among samples from New Caledonia, the Queensland coast, Torres Strait, and the Solomon Islands using the unweighted pair group method with arithmetic mean cluster algorithm and Rogers' (1972) genetic distance. Values above the nodes represent bootstrap values obtained by 1000 permutations; only values above 49.9 are given.



Genetic distances observed between *H. scabra* populations are markedly larger than in other marine invertebrates with similar larval duration (e.g., crown-of-thorns seastar (*Acanthaster planci*) (Benzie and Stoddart 1992) and giant clam (*Tridacna gigas*) (Benzie and Williams 1992)). As potential reasons for this, Uthicke and Benzie (2001) suggested the nearshore distribution of this species and scarcity of the habitat providing less opportunity for "island-hopping" than for species strongly associated with reefs. Indeed, recent studies for other coastal species in the north of Australia (barramundi (*Lates calcarifer*) (Keenan 1994; Chenoweth et al. 1998) and mudcrab (*Scylla serrata*) (Gopurenko et al. 1999)) also show very high levels of population differentiation along the coast. However, this does not explain the particularly large between-region differences in *H. scabra* (see below), but perhaps larval behaviour in the wild promotes self-recruitment, e.g., vertical migration to the benthic boundary layer within sea grass beds.

On larger geographic scales, populations from New Caledonia form a distinct cluster compared with other regions previously investigated (Uthicke and Benzie 2001), and each region is genetically distinct from the others. The closer relatedness of *H. scabra* populations from New Caledonia with populations in southern Queensland (Australia) is in concordance with recent findings of ocean surface currents (<100 m depth), which could transport larvae between the two regions (Qu and Lindstrom 2002). Likewise, surface currents form a gyre between Solomon Islands and Torres Strait (Qu and Lindstrom 2002), which may explain the con-

Fig. 3. Relationship between genetic distance and geographic distance including sample sites from New Caledonia, Knocker Bay, and Bali and previous samples from the Queensland coast, Torres Strait, and Solomon Islands. Circles indicate the following outliers (which remained in the regression analysis and Mantel (1967) test) detected by outlier analysis: W, comparison between Dungeness Reef and Warrior Reef in the Torres Straight; X, comparison between Warrior Reef and Solomon Islands; Y, comparison between Dungeness Reef and Solomon Islands; Z, comparison between Upstart Bay and Knocker Bay. The open square indicates the distinct outlier between two collection sites in the Solomon Islands (SI), the second of which was not included in the regression analysis.



nectivity in gene flow of *H. scabra* found between these two regions.

The relationship between genetic distance and the geographic distance suggests that populations of this species are in equilibrium and that gene flow is restricted and follows a pattern of isolation by distance (Slatkin 1993). This was also indicated in a previous study on *H. scabra* (Uthicke and Benzie 2001). However, that study had some gaps because no populations separated by distances from ~100 to 800 km were investigated. Data obtained here from within New Caledonia bridge this gap by providing multiple within-region comparisons and fit well into the regression. Isolation by distance was suggested for a variety of other marine species in the tropical Indo-Pacific (e.g., barramundi (Keenan 1994; Chenoweth et al. 1998), echinoids (Palumbi et al. 1997), crustaceans (Lavery et al. 1996; Benzie et al. 2002), and seastars (Williams and Benzie 1997, 1998; Benzie 1999)).

Applying the regression obtained indicates that genetic distances more than double (from 0.04–0.09) when comparing populations 100 and 500 km apart but then only double again when comparing populations 6000 km apart (Rogers' distance: 0.18). Theoretically, this regression could be used to define acceptable translocation distances for restocking purposes. However, outlier populations exist that are much more isolated than predicted by the regression, suggesting that distance alone is not a reliable proxy by which to plan translocations of *H. scabra*. From these outliers, it appears that site geomorphology is also important for gene flow and should be examined at native and nonnative sites proposed for restocking. Sites in enclosed reefs, or with eddying currents, should be excluded from broodstock collection when hatchery-produced juveniles are to be released at sites neighbouring the natal site. In addition, the sharp increase in relationship between genetic distance and geographic distance suggests caution in any translocation in this species away from the area of the native site where broodstock are collected (Hindar et al. 1991).

Similar to investigations on stock structure for wild fisheries (Waples 1995; Taylor and Dizon 1996), an acceptable level of genetic variation among populations used for restocking activities should be defined by management. If this level were to be very small (e.g., the nonsignificant Rogers' distances, ~0.02–0.04, found in this study), the statistical power of our analysis could be too small to detect them. Because only one site was sampled on the northeast coast, statistical power to detect differences to this population was presumably low and a precautionary approach would suggest that broodstock should not be collected there for release of juveniles at locations 2–4. Similarly, to maintain the integrity of populations and to avoid potential reduction in genetic diversity, animals or their progeny from sites in location 1 should not be mixed with any from the other sites.

A further issue is the appropriateness of the genetic marker to define stocks. In several cases, allozymes were suggested not to be in drift-mutation equilibrium and detection of no genetic structure may just be erroneous or represent historical patterns (Benzie 1999). Thus, in some instances, faster evolving markers such as mitochondrial DNA or microsatellites may be more appropriate and have sometimes detected differences when allozymes did not (e.g., Reeb and Avise 1990; Bowen et al. 1992; Burton 1998). However, population genetic structure for *H. scabra* in the present and previous (Uthicke and Benzie 2001) studies was detected using allozyme electrophoresis among many populations, and patterns detected suggested populations to be in equilibrium (Slatkin 1993). For these reasons, we are confident that allozymes markers are appropriate for detecting population genetic patterns in this species.

In conclusion, the findings of this research enable the planning of responsible spatial designs of restocking field experiments and future large-scale restocking of *H. scabra* for the lagoon of New Caledonia. Provided that the recommendations for broodstock numbers summarized by Utter (1998) are taken into account, we suggest that hatchery-produced juvenile *H. scabra* arising from broodstock from any of the six sites at locations 2–4 can be released into any of these sites without affecting the genetic diversity of the species in that area. However, regression analyses of the whole data set indicated that small differences in population structure might also exist among these populations.

Restocking of *H. scabra* with hatchery-produced juveniles is currently considered in a number of tropical countries. The potential for large population genetic differences between some populations separated by only small geographic distances as described here and in a previous study (Uthicke and Benzie 2001) suggests that genetic studies should be conducted in each case when hatchery-produced juveniles are relocated to areas away from the native site.

In addition to conservation reasons, such as the preservation of the natural genetic composition and diversity, population genetic differences (measured on loci presumed to be under no selection) may also suggest differences in loci related to fitness. Maintaining genetic diversity among sites safeguards against extinction (Utter 1998), for example where some stocks have high frequencies of alleles providing resilience to mortality events caused by environmental perturbations (e.g., seawater temperature fluctuations, pollutants) or disease. Moreover, because some of these alleles are adapted to local environments, it may also be that juveniles produced from those animals would have reduced fitness in other locations or regions and would therefore compromise the outcome of restocking projects. However, if stocks were overfished to the point of local extinctions, one may argue that restocking could prevent complete loss of species and would be beneficial for the health of the ecosystem, albeit with exogenous alleles. Thus, an informed risk assessment by managers may need to find compromise between loss of genetic diversity (via translocations) and loss of significant fauna (if overfished stocks fail to recover naturally).

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Macroinvertebrate response to logging in coastal headwater streams of Washington, U.S.A.

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Abstract: We examined the effects of logging on macroinvertebrate assemblages in first-order streams of four coniferous watersheds in Washington's Coastal Mountain ranges. Each watershed contained three to four first-order streams that were placed into one of three treatment types: clear-cut logging, operational buffer-strip (2.5–21 m) logging, or uncut reference streams. Prelogging baseline data on macroinvertebrate assemblages, channel morphology, sediment composition, sediment accretion rates, and water temperatures were collected from each stream in summer 1998. Logging operations were conducted the next winter and spring. Streams were resampled in summer 1999, within 1 year of logging, and summer 2000, 1+ years after logging. Preexisting treatment differences did not exist in 1998, indicating that postharvest treatment differences could be attributed to logging operations. In 1999, densities of macroinvertebrate collectors, densities and biomass of macroinvertebrate shredders, and accretion rates of organic sediments were greater in clear-cut and buffered streams than uncut references. These differences diminished by 2000. An increase in collecting and shredding macroinvertebrate is not a typical response to logging and may reflect the fact that logged streams became buried under slash, increasing detrital food supplies for these organisms. The narrow buffers used for this study did not prevent macroinvertebrate community changes associated with logging.

Résumé : Nous avons examiné les effets de la coupe des arbres sur les communautés de macroinvertébrés de ruisseaux de premier ordre dans quatre bassins hydrographiques dans la forêt de conifères des monts côtiers du Washington. Chaque bassin contenait trois ou quatre ruisseaux de premier ordre soumis à l'un des trois traitements suivants : la coupe à blanc, la coupe forestière avec maintien de bandes de protection (2,5–21 m) et aucune coupe (témoins). À l'été 1998, avant la coupe, nous avons récolté des données de base sur les communautés d'invertébrés, la morphologie du chenal, la composition des sédiments, les taux d'accumulation des sédiments et la température de l'eau de chacun des ruisseaux. La coupe s'est faite l'hiver et le printemps suivants. Nous avons échantillonné les ruisseaux à nouveau à l'été 1999, soit pendant l'année qui a suivi la coupe, ainsi qu'à l'été 2000, 1+ année après la coupe. Comme en 1998, avant les travaux forestiers, il n'y avait pas de différences entre les ruisseaux, les différences observées après la coupe pouvaient être attribuées aux divers traitements expérimentaux. En 1999, les densités des macroinvertébrés collecteurs, les densités et la biomasse des macroinvertébrés déchiqueteurs et les taux d'accumulation des sédiments étaient tous supérieurs dans les ruisseaux soumis à la coupe à blanc et à la coupe avec maintien de bandes de protection que dans les ruisseaux témoins non soumis à la coupe. Ces différences étaient moins accentuées en 2000. Un accroissement des populations d'invertébrés collecteurs et déchiqueteurs n'est pas un effet habituel de la coupe forestière et s'explique peut-être par le fait que les ruisseaux soumis à la coupe ont été couverts de débris ligneux, ce qui a augmenté la quantité de détritus qui sert de nourriture à ces organismes. Les étroites bandes de protection utilisés dans notre étude n'ont pas empêché les changements dans les communautés de macroinvertébrés associés à la coupe forestière.

[Traduit par la Rédaction]

Introduction

Headwater streams can be strongly influenced by land-use practices on adjacent land because of the tight ecological linkages between channels and riparian zones (litter inputs, shading) (Vannote et al. 1980; Vuori and Joensuu 1996). Commercial timber harvesting can affect supplies of sediment and woody debris to streams, which partly controls channel structure, and channel irradiance and water temperatures can be affected if riparian shading is reduced (Murphy and Hall 1981). In the Coastal Mountains of the Pacific Northwest, U.S.A., commercial forests of second- or third-growth timber cover most of the headwater landscape (Brown and Krygier 1971). Despite the intensive nature of logging in this area, minimal information is available about impacts of logging on regional headwater streams.

Many states in the United States have regulations requiring riparian buffers to minimize impacts of logging on streams. Riparian buffers can stabilize stream banks, provide shade, supply woody debris and organic debris to channels, and filter overland flows (Vannote et al. 1980; Gregory et al. 1991; Belt and O'Laughlin 1994). However, for logging operations typically practiced in Washington's Coastal Mountains during the 1990s, it was not considered necessary to leave buffer strips to protect small headwater streams be-

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cause these habitats did not support fish (Washington Department of Natural Resources Forest Practices Division 1993). However, concerns have developed about impacts on other resident fauna, particularly amphibians, as well as the implications of impacts in the headwaters on downstream portions of watersheds.

This study was designed to assess the effects of logging on macroinvertebrate assemblages of perennial first-order streams of Washington's Coastal Mountains and to determine whether riparian buffer strips were effective at minimizing change. Stream macroinvertebrates can be useful bioindicators to assess effects of logging because many taxa are sensitive to changes in sediment, organic material, temperature, and light (Newbold et al. 1980; Hachmöller et al. 1991; Brown et al. 1997).

Materials and methods

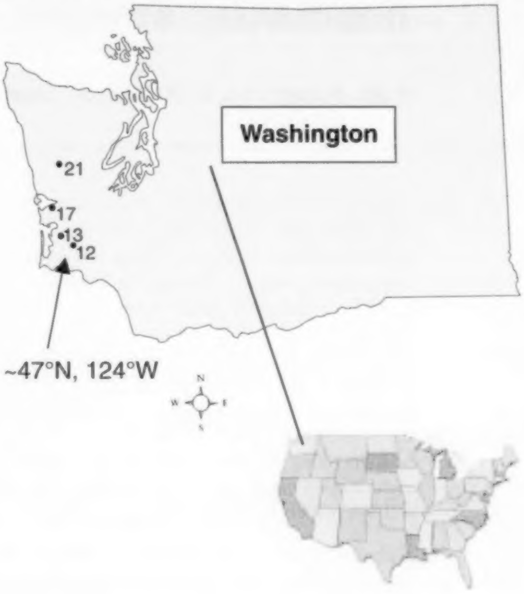
Site description

We studied perennial first-order streams within four watersheds of coastal Washington State (Fig. 1). These watersheds were selected because they contained three or more first-order streams within a harvest unit scheduled for logging in 1999. Pacific County Watershed (WS) 12 and Pacific County WS 13 were located in the Willapa Hills outside Menlo and South Bend, respectively. Grays Harbor County WS 17 was located outside Aberdeen, on the south shore of Grays Harbor. Grays Harbor County WS 21 was near the southwest corner of Olympic National Park. Watershed numbers refer to township designations of the U.S. land survey.

Watersheds were located in a region with wet, mild winters and warm, dry summers. Average annual precipitation over the four sites ranged from 2030 to 3300 mm·year^{-1} due to orographic differences, and mean monthly air temperatures ranged from a low of 1.0 °C in January to 17.4 °C in August. The watersheds were small (15.9–53.5 ha), with streams having narrow and steep channels (0.9- to 3.6-m active channel widths and 8–30% channel slopes) (Jackson and Sturm 2002). Gravel and bedrock outcrops dominated the channel beds. Lithology and topography varied among the four sites (Haggerty 2000). Numerous woody debris dams created a step–riffle habitat in most streams (Jackson et al. 2001; Jackson and Sturm 2002). All sites were dominated by 50- to 65-year-old second-generation-growth western hemlock (*Tsuga heterophylla*) with a minor component of western redcedar (*Thuja plicata*). The understory was dominated by salmonberry (*Rubus spectabilis*), devils club (*Oplopanax horridum*), salal (*Gauftheria shallon*), and sword fern (*Nephrolepis* sp.). Additional description of the study watersheds is available in Jackson et al. (2001), Haggerty et al. (2002), and Jackson and Sturm (2002).

Baseline sampling of the invertebrate assemblages in the first-order streams of theses watersheds (Haggerty et al. 2002) indicated that 31 insect and four noninsect taxa (genera, subfamilies, or families) occurred representing nine orders and all functional feeding groups. Assemblages were numerically dominated by Gammaridae (Amphipoda), Chironomidae (Diptera), Elmidae (Coleoptera), Nemouridae (Plecoptera), Empididae (Diptera), and Rhyacophilidae (Trichoptera). Astacidae (Decapoda), Elmidae, Gammaridae, and Perlidae (Plecoptera) contributed most to the total biomass. Baseline studies indicated that invertebrate assemblages differed somewhat among watersheds (Haggerty et al. 2002), but streams within each individual watershed were similar and could be viewed as blocked replicates.

Fig. 1. Map of Washington, U.S.A., indicating the locations of each of the four study watersheds.



Logging treatments

After baseline data were collected in summer 1998, streams within each of the four study watersheds were assigned, randomly where possible, to one of three treatment types: clear-cut logging (two streams in WS 12, two in WS 13, two in WS 17, and one in WS 21), buffer-strip logging (one in WS 13, one in WS 17, and two in WS 21), and uncut reference streams (one in each watershed). Clear-cuts extended to the stream bank, and all riparian trees were removed. Buffers were created by the commercial loggers harvesting the sites using operational techniques based on side slope (topography), tree type, and yarding considerations. Buffer widths varied from 2.5 m at the narrowest points to 21.0 m at the widest. All logging of the study watersheds took place between November 1998 and April 1999. Reference streams had no vegetation removed in their catchments and these habitats had not been logged for 50–65 years. On WS 12, WS 13, and WS 17, all of the contributing area outside the buffer was clear-cut. On WS 21, a logging road bisected the contributing basins, and the upper halves of the basins were not harvested.

The main types of logging used were highlead and skyline logging (Smith 1986), which is typical for coastal Washington. These logging techniques require the use of portable steel towers, which connect wire cables to notched stumps on opposite hillsides. The cables assist in removal of cut trees from a site and stabilize the tower (Wenger 1984). All trees are felled by hand in the down-slope direction. Upon cutting, trees are limbed and topped. All residual slash is left on site. This slash and other logging debris tend to move down slope and accumulate in stream channels, often burying the streams under 0.5–2 m of material (Froehlich 1973; Jackson et al. 2001). Log boles are attached to skidder lines (cables) and dragged upslope to a landing where they are loaded onto trucks. Trees are often hauled in the same direction upslope, creating localized bare-soil skid trails that are susceptible to erosion. After logging, wind throw frequently knocked down trees in remnant buffers, and between 33% and 64% of the trees in the study buffers had toppled by summer 2001 (unpublished data).

Sampling

In the summer of 1998, the baseline year, and in the summer of 1999, the first growing season after harvest, we sampled all 15 study streams. In summer 2000, we sampled only 12 of the 15 streams (six clear-cut, three reference, and three buffered); that year, one reference was logged, and one clear-cut and one buffered stream went dry, so those three streams were not sampled.

Macroinvertebrate assemblages in each stream were sampled using substrate baskets. We selected these samplers because unlike more standard samplers (Surbur nets, corers, kick nets), they could be used efficiently in both natural and slash-covered streams. Baskets (30 cm × 30 cm) were fashioned from chicken wire mesh (2.5-cm mesh size) and were filled with 1.5 L of wood and 1.5 L of cobble (average surface area for wood was 283 cm^2 and for cobble was 153 cm^2). Wood and cobble were gathered directly from the channel so natural substrate conditions were present (i.e., biofilms). In June, baskets (three in each reference stream and four in each buffered and clear-cut stream) were installed into pools and were left for 6 weeks. Baskets were placed into pools because riffles or runs were too shallow to cover the baskets, but they were placed at pool inlets to ensure that a range of flow conditions was sampled. Basket placement in clear-cut streams in 1999 and 2000 was stratified so that two baskets were placed in pools open to sunlight and two in pools covered by slash. In the latter case, we dug down through the slash, placed the basket, and then replaced the slash. However, because we did not detect differences between open and slash-covered baskets (Haggerty 2000), these four subsamples were pooled for analyses.

In August, baskets were removed from pools and immediately transferred into buckets. As flows are at their lowest during this time of year, it was feasible to place buckets in the channel and quickly transfer the basket into the bucket, thereby minimizing loss of invertebrates. Samples were partially processed on site to reduce the bulk of material that would need to be transported from the remote sites set in steep terrain. First, woody debris was removed from the basket and placed into plastic bags. Then, cobble was washed and scrubbed and the slurry was passed through a 1-mm-mesh net and sample residue was added to the bags with the woody debris. Calibration studies (Haggerty 2000) found that field sieving of samples resulted in the loss of 17% of the invertebrates, consisting almost exclusively of small nemourids, chironomids, and amphipods. Appropriate correction factors were used when calculating those macroinvertebrate abundances. Organisms passing through the sieve were very small and comprised only 0.2% of a sample's invertebrate biomass, and so correction for biomass was unnecessary.

All samples were preserved in the field with 95% ethanol and transferred back to the laboratory where macroinvertebrates were identified to genus, when possible, using keys in Merritt and Cummins (1996), Stehr (1991), and Stewart and Stark (1993). Chironomid larvae were mounted and identified to subfamily using Epler (1995). Macroinvertebrate taxa richness (based on the lowest taxonomic level used), abundance, and biomass were calculated for each sample. Biomass was estimated by measuring macroinvertebrates to the nearest 1 mm and converting lengths to biomass (ash-free dry mass) using length–weight regression equations provided in Benke et al. (1999). Macroinvertebrates were categorized into functional feeding groups (shredders, collectors, scrapers, and predators) following Merritt and Cummins (1996).

Geomorphology

Physical channel surveys were conducted before harvest to provide a baseline from which to measure physical channel changes after treatment. Surveys were conducted in the same reach of stream where biological sampling was done, and each channel survey extended upstream approximately 20 channel widths, to the end of the channel, or to an obvious break in overall channel morphology. The first-order streams in our study areas were quite short. In some cases, the total length of a stream was less than 100 m. We measured the length, width, and height or drop of each channel habitat unit. The common channel habitat units include riffles, pools, steps, subsurface flow, bedrock cascades, and runs. Particle size distributions were determined from substrate samples collected at three randomly selected locations to determine percent fines (Corner et al. 1996).

Rock tiles (unglazed marble, 12.5 cm × 7.5 cm) were installed adjacent to substrate baskets to measure organic and inorganic matter accretion in stream reaches before and after logging. Upon removal in August, tiles were lifted straight out of the water, placed into plastic bags, and frozen until processed. Sediment was removed, dried at 60 °C for 24–48 h, weighed to the nearest milligram, ashed in a muffle furnace, and reweighed to determine ash-free dry mass and ash mass. The ash-free dry mass was used as an index of organic matter accumulation (detritus, aufwuchs) and the ash mass was used as an index of inorganic sediment accretion.

Stream temperature

Temperatures were measured using HOBO7 XT temperature loggers that were programmed to collect temperatures on an hourly basis. The cases were set in the bed of the streams, usually in 5–10 cm of water, with rocks placed on top of the cases to prevent them from washing away. During both the pre- and post-harvest summers, temperature loggers were placed in streams in early to mid-June and removed in early August.

Statistical analyses

Two-way (watershed × logging treatment) analysis of variance (ANOVA) followed by a Tukey–Kramer post hoc mean comparisons test was used on the 1998 baseline data to verify that streams assigned to the different logging treatments were similar, and permitting us to infer that any postharvest differences that developed were related to harvest technique. The data from 1999 (less than 1 year after harvest) and 2000 (1 year later) were analyzed in the same manner. Analyses were conducted using SuperANOVA software (Abacus Concepts, Berkeley, Calif.); this program uses general linear modeling permitting analyses of unbalanced data sets. Dependent response variables for these analyses included (*i*) overall macroinvertebrate taxa richness, abundance, and biomass, (*ii*) abundance and biomass of individual functional feeding groups, (*iii*) abundance of individual taxa, and (*iv*) organic and inorganic sediment accretion. Crayfish posed problems for statistical comparisons of biomass because their mass was large but it resulted from only a few individuals. Thus, although crayfish were included for analyses of macroinvertebrate richness and abundance, crayfish were not considered for biomass comparisons among logging treatments. For each variable of interest, subsamples within streams were pooled into a single measure per year and that value was used for statistical tests (i.e., whole streams were our analytical units). Before analyses, conformity to ANOVA assumptions was assessed using the procedures for two-way tests described in Snedecor and Cochran (1967). The presence of gross outliers was assessed by inspection, and indicators of nonnormality (coefficients of variation >1) or unequal variance (dependency of the variance on the mean) were assessed by examining relationships between sample means and error terms. If data possessed characteristics of nonconformity, data were transformed ($\log_{10}(x + 1)$ or $\sqrt{x}$) as recommended by Snedecor and Cochran (1967). After transformation, relationships between means and error terms were reassessed to determine if transformed data more closely approximated normal distributions with near equal variance. Analyses where significant interaction existed between the main effects (watershed and logging treatment) were not interpreted.

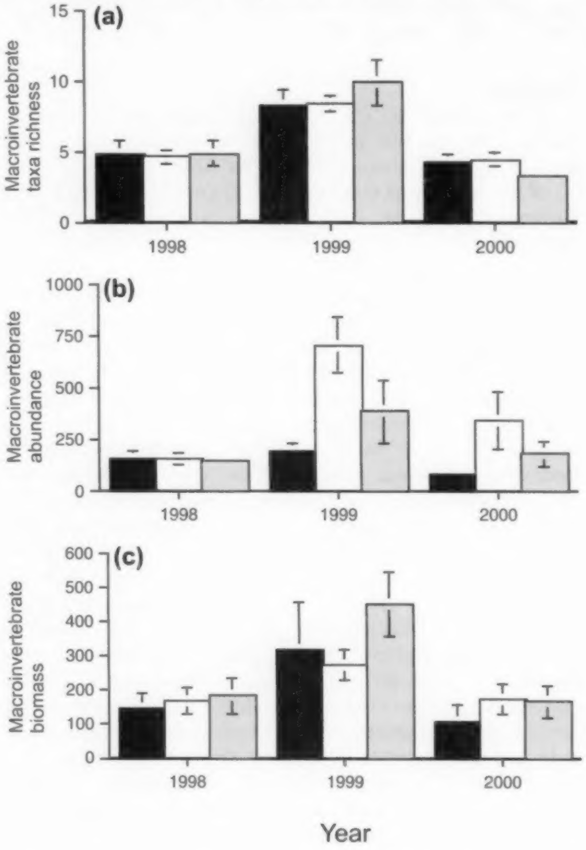
From the hourly temperature observations, daily maximum temperatures were calculated for the preharvest summer of 1998 and the postharvest summer of 1999. The daily maximum stream temperatures were then plotted against the appropriate reference stream to create regression equations. The slopes of the pre- and post-harvest regression lines were compared using a Student's *t* test. This test allowed us to determine if pre- and post-harvest years had significantly different slopes.

Results

Macroinvertebrates

In 1998, before any harvest, streams assigned to be references, clear-cut, or to be harvested with buffers did not differ in terms of their overall invertebrate richness, density, or biomass (Fig. 2) or their density and biomass of individual functional feeding groups (Fig. 3) or individual taxa. For some analyses, differences among the four watersheds were detected, and thus, we retained blocking streams by watershed for all subsequent tests.

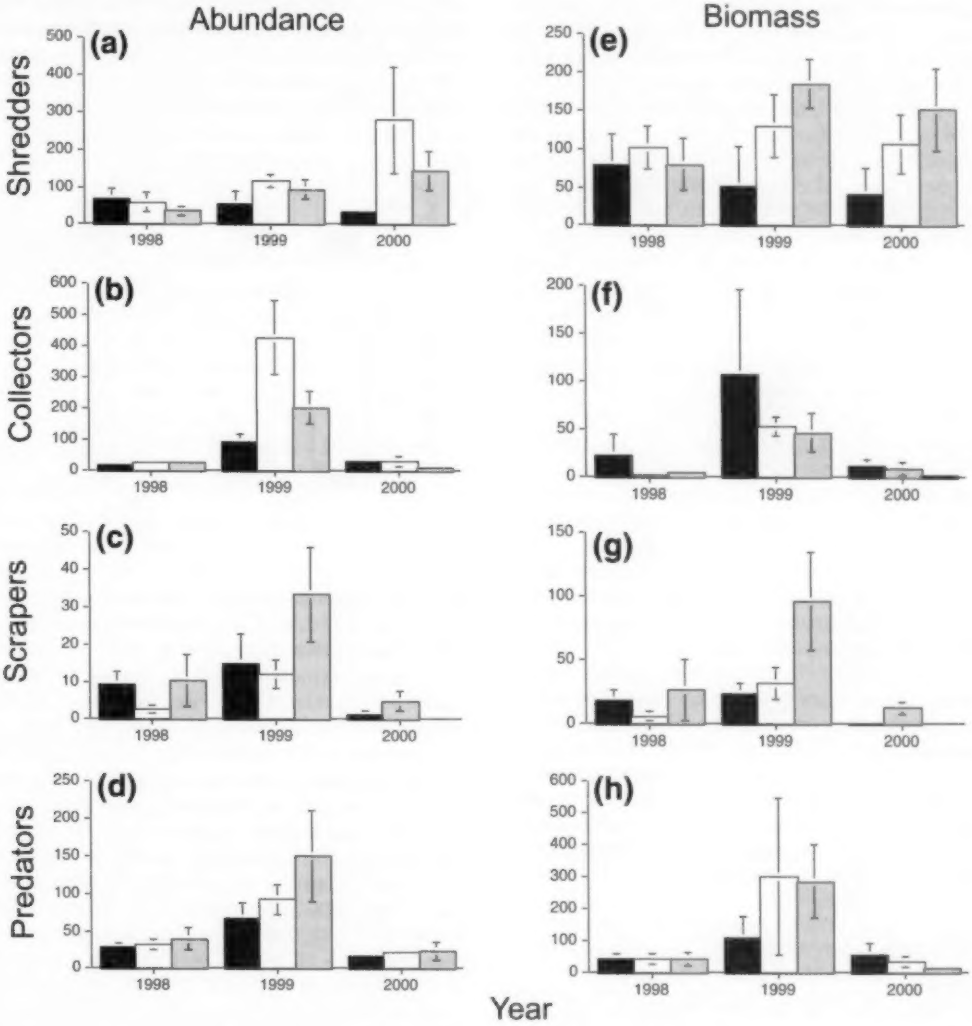
Fig. 2. Macroinvertebrate (*a*) taxa richness (taxa per basket sample), (*b*) abundance (invertebrates·m^{-2}), and (*c*) biomass (excluding crayfish; mg AFDM·m^{-2}) in reference (solid bars), clear-cut (open bars), and buffered (shaded bars) streams in 1998, 1999, and 2000. Substrate basket samplers (30 cm × 30 cm) were used for macroinvertebrate collection. Error bars indicate ±1 SE. Invertebrate measures were similar among treatments in all cases except that during 1999, clear-cut streams supported significantly more macroinvertebrates compared with reference streams.



In 1999, less than 1 year after harvest, invertebrate taxa richness did not vary among streams subjected to the different management techniques (Fig. 2). However, overall invertebrate densities were greater in clear-cut streams than in reference streams ($F_{[2,4]} = 11.1$, $P = 0.023$) (Fig. 2), with densities in buffered streams being intermediary. Differences in density were driven by the responses of two functional feeding groups (Fig. 3), shredders ($F_{[2,4]} = 15.9$, $P = 0.013$) and collectors ($F_{[2,4]} = 17.5$, $P = 0.011$). Shredder densities were greater in both clear-cut and buffered streams than in the reference streams. Collector density was greatest in the clear-cut streams and lowest in the reference streams. Densities of scrapers and predators did not significantly differ among treatments. Densities of individual taxa were all similar among treatments (Appendix A).

Overall invertebrate biomass did not significantly differ among treatments in 1999 (Fig. 2). However, shredder biomass was greater in the clear-cut and buffered streams than in the reference streams ($F_{[2,4]} = 19.7$, $P = 0.009$) (Fig. 3). Predator biomass also differed marginally among treatments ($F_{[2,4]} = 7.3$, $P = 0.045$), but this result was not interpreted because a significant interaction ($P = 0.029$) existed between

Fig. 3. (*a–d*) Abundance (organisms·m^{-2}) and (*e–f*) biomass (mg AFDM·m^{-2}) of shredders, collectors, scrapers, and predators in reference (solid bars), clear-cut (open bars), and buffered (shaded bars) streams in 1998, 1999, and 2000. Error bars indicate ±1 SE. Significant differences in abundance among treatments existed for shredders in 1999, where densities in clear-cut and buffered streams exceeded densities in reference streams, and for collectors in 1999, where densities in clear-cut streams exceeded densities in reference streams. Significant differences in biomass among treatments existed for only shredders in 1999, where biomass was greater in clear-cut and buffered streams than in reference streams.



harvest treatment and watershed. Interaction was not detected for any other test. Collector and scraper biomass differ not differ significantly among treatments.

By 2000, over 1 year after harvest, the differences detected in 1999 were no longer significant and no new patterns emerged. Biological and procedural changes probably both contributed to the differences in response between 1999 and 2000. Collector numbers (mostly chironomid midges) decreased in all habitats from 1999 to 2000 (Appendix A; Fig. 3) (Haggerty 2000), which likely contributed to the lack of response in 2000. Patterns in shredder biomass among treatments in 2000 visually appeared to mirror the pattern of 1999 (Fig. 3), but treatment differences were no longer significant. The reduced level of response might have been due to a dampening of impacts, or it could have resulted from reduced statistical power in 2000 because three streams were lost from the study that year (one reference stream was harvested and two other streams were too dry to sample).

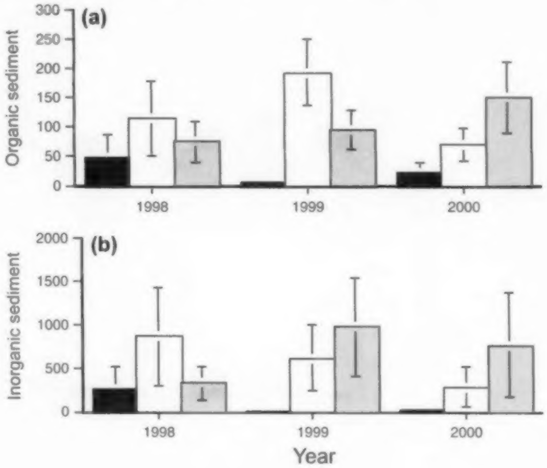
Geomorphology and stream temperature

A detailed analysis of channel morphology and stream temperature changes is presented in Jackson et al. (2001). Jackson et al. reported that, after logging, 48% of the clear-cut channel length was covered by logging debris (i.e., a recognizable channel still existed below the debris) and 50% of the clear-cut channel length was buried (i.e., flow moved through a matrix of organic debris and sediment without a recognizable channel). The average depth of coverage and burial was 0.97 m. The logging debris trapped fine sediments in the channels, increasing the percentage of fines from an average of 12% to 44% (Jackson et al. 2001).

In the pretreatment and reference streams, summer water temperatures varied from 7 to 16 °C. Some streams were fed apparently by relatively deep groundwater flow, and in these streams, temperatures showed little diurnal or seasonal variation (less than 2 °C variation over the summer). Even with >95% canopy cover, other streams exhibited diurnal variations up to 2 °C and variation over the summer of 5 °C. Harvest effects on maximum daily temperatures were assessed by looking at shifts in regressions between treatment and reference streams (Jackson et al. 2001). In the seven clear-cut streams, there were no consistent shifts in stream temperatures in relation to reference streams because the slash covering the channel acted as an effective thermal buffer. In three of the seven streams, there was no statistically significant shift in temperatures. In two of the clear-cut streams, temperatures changed less than 2 °C (one increase and one decrease), and in the remaining two streams, temperature shifts depended on the location of the temperature loggers. For example, one location showed an increase of 16.8 °C, whereas another location in the same stream experienced a 2.5 °C reduction in temperature after harvest. Temperatures in two of the three buffered streams increased slightly (2–2.6 °C), and temperatures in the nonmerchantable buffer streams increased 2.8–4.9 °C, apparently because of reduction in canopy cover.

Before any logging in 1998, organic and inorganic matter accretion on rock tiles was similar among projected treatments (Fig. 4). In 1999, less than 1 year after logging, accretion of organic matter on tiles was significantly greater ($F_{[2,4]} = 83.3$, $P = 0.0005$) (Fig. 4) on tiles in clear-cut and buffered streams than on tiles in reference streams. However, accretion of inorganic matter did not differ significantly among treatments that year ($P = 0.0720$). In 2000, the accretion of organic matter on tiles was again greater in buffered streams that in the reference streams ($F_{[2,2]} = 18.6$, $P = 0.0511$) (Fig. 4), but clear-cut and reference streams did not differ that year. Accretion of inorganic matter did not differ among treatments in 2000 ($P = 0.7354$).

Fig. 4. (*a*) Organic (mg AFDM·m^{-2}) and (*b*) inorganic (mg ash·m^{-2}) sediment accretion on stone tiles placed in reference (solid bars), clear-cut (open bars), and buffered (shaded bars) streams in 1998, 1999, and 2000. Error bars indicate ±1 SE. Significant differences among treatments existed only for organic sediments in 1999, where accretion in clear-cut and buffered streams exceeded accretion in reference streams, and organic sediments in 2000, where accretion in buffered streams exceeded accretion in reference streams.



Discussion

Impacts of logging

In coastal Washington, we detected ecological responses to logging that were different from those reported in streams elsewhere. Typically, logging shifts the macroinvertebrate assemblage towards one that is scraper based. Normally, riparian vegetation limits algal production in headwater streams through light attenuation (Gregory et al. 1991). Clear-cut logging causes headwater systems based on allochthonous input of plant detritus (leaves and wood) to become based instead on autochthonous growth of algal periphyton (Murphy and Hall 1981; Duncan and Brusven 1985). Stimulating periphyton growth then increases the density and biomass of scrapers (Murphy et al. 1981; Silsbee and Larson 1983; Hachmöller et al. 1991). However, in coastal Washington, scrapers did not respond to logging and were, in fact, a very small portion of the benthos throughout the study. Even in areas open to sunlight, clear-cut logging did not induce a scraper response. Instead, we found that collectors and shredders, rather than scrapers, were the groups most affected by logging, at least over the short term.

A second typical impact of logging on stream channels, which is also related to the loss of riparian shading, is an increase in water temperatures (Brosofske et al. 1997). After the removal of the canopy cover from adjacent forests, insulation of channels increases, as well as heat exchange, because of the loss of the windscreen. However, we did not observe a postharvest increase in temperature in clear-cut streams. Stream temperatures were similar in clear-cut, buffered, and reference streams, presumably because groundwater discharge is the main source of flow or because slash protected the channel from solar radiation or acted as thermal insulation in the clear-cut streams.

A reduction in the input of large woody debris is yet another typical postlogging impact on streams (Murphy and Hall 1981; Hilderbrand et al. 1997). However, in coastal Washington, rather than a decrease in wood loading, we observed a dramatic increase in woody debris in logged streams. The process of tree felling and the subsequent limbing and topping of trees near stream channels, combined with the steep slopes, caused slash to accumulate in channels, in some cases completely burying them.

The primary influences of logging measured by this study were increases in invertebrate collector densities, invertebrate shredder densities and biomass, organic matter accretion, and slash cover. Although the invertebrate responses detected in this study were unique, they were still probably food related, as is the case elsewhere. But instead of being affected by changing algal supplies, invertebrates in coastal Washington were probably responding to changing detrital supplies. The increase in shredder density and biomass in logged streams was probably caused by the influx of wood into the streams. Wood is known to be an important resource for shredding invertebrates in west coast streams (Anderson et al. 1978; Pereira et al. 1982; Haggerty et al. 2002). The observed increase in collector numbers in 1999 was probably related to the increase in organic matter accretion on surfaces. Chironomids dominated the collector group in this study and these organisms use organic-rich sediments as food and habitat (Newbold et al. 1980; Brown et al. 1997). All of these responses appeared to being waning by 2000, and we anticipate that the study streams might respond more like streams elsewhere after slash decays or is mobilized during high flows and stream channels become exposed.

Efficacy of buffer strips

The buffer strips used in this study performed poorly, at least in terms of the factors that we measured. Many of the changes in macroinvertebrate abundance and biomass and organic matter accretion caused by clear-cutting occurred in similar fashion in the buffered streams. For organic matter accretion, the change was more prolonged in buffered streams than in clear-cut streams. We suspect that the buffers in this study were too narrow (2.5–21 m) to be effective. In fact, the toppling of exposed buffer trees to wind throw may have affected sediment dynamics and could cause bank stability problems in the future. Since the inception of this project, Washington's forest practice rules have been changed and now require 15-m no-cut buffers on 50% of the length of non-fish-bearing streams, with a minimum buffered length of 90 m and a maximum buffered length of 150 m. These buffered areas must include seeps, springs, and channel confluences.

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Appendix A

Table A1. Densities (no.·m^{-2} (±1 SE in parentheses)) of macroinvertebrates collected from perennial first-order streams in four study watersheds of Washington's Coastal Mountains, U.S.A., in 1999 and 2000.

		1999			2000		
Taxon	FFG[a]	Clear-cut	Buffered	Reference	Clear-cut	Buffered	Reference
Gastropoda							
Pleuroceridae							
Juga	Sc	2.43 (1.56)	12.03 (7.17)	3.33 (3.33)	0.47 (0.47)	0	0
Oligochaeta							
Tubificidae	C	11.80 (2.33)	6.48 (3.22)	7.78 (2.37)	2.78 (1.92)	0.92 (0.92)	3.67 (2.62)
Amphipoda							
Gammaridae							
Gammarus	Sh	46.66 (17.0)	82.55 (30.77)	35.11 (30.77)	194.44 (79.99)	105.55 (49.33)	6.18 (4.89)
Decapoda							
Astacidae							
Pacifastacus	P	0.34 (0.34)	1.86 (1.36)	0	1.39 (1.39)	0	1.23 (1.23)
Ephemeroptera							
Baetidae							
Paracloeodes	Sc	1.39 (0.66)	4.63 (2.56)	6.67 (3.78)	0	0	0
Heptageniidae							
Ironodes	Sc	5.55 (2.34)	1.86 (1.86)	6.67 (2.97)	0.47 (0.47)	0	0
Leucrocuta	Sc	2.78 (1.73)	13.89 (5.82)	0	0	0	0
Leptophlebiidae							
Paraleptophlebia	C	4.17 (4.17)	0	0	0	0	0
Plecoptera							
Capniidae							
Paracapnia	Sh	2.10 (1.67)	0	0	1.86 (1.09)	0	0
Chloroperlidae							
Haploperla	P	5.55 (2.96)	0.92 (0.92)	2.22 (2.22)	0	0	0
Neaviperla	P	3.70 (0.79)	1.86 (1.36)	18.89 (11.10)	0	0	0
Sweltsa	P	9.38 (2.50)	10.19 (5.88)	0	0	0	0
Leuctridae							
Despaxia	Sh	4.89 (1.87)	18.62 (7.05)	16.66 (10.43)	10.70 (3.24)	0	6.18 (1.96)
Paraleuctra	Sh	2.78 (2.78)	1.86 (1.24)	0	0	0	0
Nemouridae							
Nemoura	Sh	0	0.92 (0.92)	0	7.41 (6.11)	0.92 (0.92)	0
Soyedina	Sh	21.62 (10.12)	2.89 (2.89)	0	45.77 (26.55)	5.67 (4.63)	16.89 (3.78)
Zapada	Sh	28.54 (9.47)	15.00 (7.33)	22.22 (7.00)	13.89 (6.90)	1.89 (1.89)	11.11 (8.55)
Peltoperlidae							
Soliperla	Sh	1.04 (0.58)	0.92 (0.92)	4.44 (2.22)	0	0	0
Yorapera	Sh	2.09 (1.76)	0	2.22 (1.48)	1.39 (0.77)	0	1.22 (1.22)
Perlidae							
Calineuria	P	0	0	1.11 (1.11)	0	0	2.44 (1.67)
Doroneuria	P	0	0	3.33 (1.70)	1.86 (1.09)	0	0

Table A1. (*concluded*)

Taxon	FFG[a]	1999			2000		
		Clear-cut	Buffered	Reference	Clear-cut	Buffered	Reference
Perlodidae							
Isoperla	P	0.34 (0.34)	0	0	0	0	0
Rickera	P	1.04 (0.77)	1.86 (1.24)	0	0	0	0
Taeniopterygidae							
Doddsia	Sc	0.34 (0.34)	0	0	0	0	0
Trichoptera							
Brachycentridae							
Eobrachycentrus	Sh	0	0.92 (0.92)	0	0	0	0
Hydropsychidae							
Parapsyche	C	0	0	7.78 (5.51)	0	0	0
Lepidostomatidae							
Lepidostoma	Sh	0	1.86 (1.36)	0	0	0	0
Limnephilidae							
Hydatophylax	Sh	0.34 (0.34)	0	2.22 (1.48)	0.92 (0.64)	0	2.44 (2.44)
Psychoglypha	C	1.04 (0.77)	4.63 (3.20)	1.11 (1.11)	0	0	0
Psychomyiidae							
Psychomyia	C	1.39 (0.58)	1.86 (1.36)	4.44 (3.4)	0	0	0
Rhyacophilidae							
Himalopsyche	P	1.04 (0.58)	6.48 (3.20)	0	0	0	0
Rhyacophila	P	7.99 (2.44)	19.44 (8.44)	17.78 (8.55)	8.8 (2.77)	6.48 (4.82)	1.22 (1.22)
Coleoptera							
Dryopidae							
Helichus	Sh	1.39 (0.66)	0.92 (0.92)	0	0	0.92 (0.92)	0
Elmidae							
Lara	Sh	7.66 (2.66)	10.20 (3.98)	2.22 (1.44)	3.24 (1.24)	17.61 (8.55)	2.44 (1.63)
Psephenidae							
Acneus	Sc	1.98 (0.89)	0.92 (0.92)	5.55 (3.78)	0.92 (0.64)	0	1.22 (1.22)
Diptera							
Athericidae							
Atherix	P	1.39 (0.66)	1.86 (1.86)	0	0	0	0
Ceratopogonidae							
Bezzia	P	0.69 (0.48)	0	0	1.39 (1.02)	0.92 (0.92)	1.22 (1.22)
Ceratopogon	P	0.69 (0.48)	0	0	0		
Chironomidae							
Chironominae	C	82.23 (16.55)	66.66 (30.86)	13.33 (5.5)	8.80 (5.09)	3.67 (2.09)	1.22 (1.22)
Diamesinae	C	30.11 (7.87)	10.19 (5.71)	22.22 (2.22)	0	3.67 (3.67)	2.44 (2.44)
Orthocladinae	C	264.40 (72.94)	115.66 (31.44)	64.44 (9.44)	16.22 (6.66)	2.78 (2.78)	20.89 (8.33)
Tanypodinae	P	36.20 (7.88)	79.55 (37.55)	3.33 (2.37)	6.02 (3.28)	1.86 (1.24)	0
Dixidae							
Dixa	C	15.99 (5.89)	36.11 (17.54)	7.78 (3.78)	2.31 (0.94)	0.92 (0.92)	4.89 (3.78)
Empididae							
Chelifera	P	12.19 (4.33)	8.33 (4.37)	7.78 (2.38)	2.31 (0.94)	10.19 (3.16)	4.89 (3.78)
Pelecorhynchidae							
Glutops	P	0.69 (0.48)	0	2.22 (2.22)	0	0.92 (0.92)	2.44 (1.67)
Psychodidae							
Pericoma	C	0	0	0	0	0.92 (0.92)	0
Tipulidae							
Dicranota	P	1.39 (0.97)	3.67 (3.67)	0	0.92 (0.64)	0	1.22 (1.22)
Hexatoma	P	5.55 (1.93)	2.78 (1.99)	3.33 (2.37)	0.47 (0.47)	0.92 (0.92)	1.22 (1.22)
Pedicia	P	0	0.92 (0.92)	0	0	0.92 (0.92)	0
Sciaridae							
Sciara	C	1.04 (0.58)	0.92 (0.92)	2.22 (1.44)	0	0	0

Note: Substrate basket samplers (30 cm × 30 cm, 1.5 L of wood and 1.5 L of cobble) were used to sample macroinvertebrates.

[a] Functional feeding group: C, collectors; P, predators; Sc, scrapers; Sh, shredders.

Impacts of sediment dewatering and rehydration on sediment nitrogen concentration and macrophyte growth

William F. James, John W. Barko, and Harry L. Eakin

Abstract: River sediments were experimentally dewatered via exposure to air from an initial moisture content of 75% to 61% (~20% dewatered), 32% (~60% dewatered), and 4% (~95% dewatered). Sediments dewatered by 20% and 60% and rehydrated exhibited a lower exchangeable ammonium-N (NH_4-N) concentration and a lower rate of nitrate/nitrite-N and NH_4-N release from sediments compared with controls. In contrast, sediments dewatered by 95% and rehydrated exhibited a marked increase in exchangeable sediment NH_4-N and a much higher rate of nitrate/nitrite-N (under oxic conditions) and NH_4-N (under oxic and anoxic conditions) release from sediments. Sediment dewatered by 60% and 95% and rehydrated lost total N (18%), suggesting denitrification. Growth of *Potamogeton pectinatus* responded to changes in the exchangeable sediment NH_4-N concentration; it was lower on partially dewatered and rehydrated sediments that exhibited lower exchangeable sediment NH_4-N and elevated on sediment that was dewatered by 95%. Our results suggest that sediment N may be altered via sediment dewatering and rehydration to affect macrophyte growth.

Résumé : Nous avons déshydraté expérimentalement par exposition à l'air des sédiments de rivière dont le contenu initial d'humidité était de 75 % à 61 % (~20 % de déshydratation), 32 % (~60 % de déshydratation et 4 % (~95 % de déshydratation). Les sédiments déshydratés de 20 % et de 60 % et ensuite réhydratés possèdent une concentration réduite d'azote-ammonium (NH_4-N) échangeable et un taux de libération réduit d'azote-nitrate/nitrite et de NH_4-N par comparaison aux sédiments témoins. En revanche, les sédiments déshydratés de 95 % et ensuite réhydratés affichent un accroissement marqué de NH_4-N échangeable dans les sédiments et des taux beaucoup plus élevés de libération d'azote-nitrate/nitrite (en conditions oxiques) et de NH_4-N (en conditions oxiques et anoxiques). Les sédiments déshydratés de 60 % et de 95 % et ensuite réhydratés perdent 18 % de leur azote total, ce qui fait croire à une dénitrification. La croissance de *Potamogeton pectinatus* réagit aux changements de concentrations du NH_4-N échangeable dans les sédiments; elle est plus faible dans les sédiments déshydratés puis réhydratés qui possèdent des concentrations plus basses de NH_4-N échangeable et plus élevée dans les sédiments déshydratés à 95 %. Nos résultats indiquent que l'azote des sédiments peut être altéré par la déshydratation et la réhydratation de manière à affecter la croissance des macrophytes.

[Traduit par la Rédaction]

Introduction

Evidence is strong that macrophyte communities play a critical role in the structure and function of aquatic ecosystems (Jeppesen et al. 1998*a*; Scheffer 1998 and references therein). They influence food web dynamics by providing refugia for small fish and zooplankton populations against predation, which results in increased survival rates and elevated grazing pressure on pelagial phytoplankton (Timms and Moss 1984; Jeppesen et al. 1998*b*). Extensive littoral surface area in the form of macrophyte structure supports a proliferation of epiphytic and macroinvertebrate communities that provide food sources to sustain higher trophic levels (Diehl and Kornijów 1998; Jones et al. 1998). Macrophytes also stabilize the sediments from erosion and focusing to deeper regions (James and Barko 1990) and minimize sediment resuspension and high turbidity in the water column by dampening wave activity, creating a clear-water state (James and Barko 1994; Barko and James 1998). Carbon sequestered by macrophytes and associated epiphyton also supports energy flow through detrital pathways, leading to greater diversity of alternative food resources for higher trophic levels (Rich and Wetzel 1978).

Scheffer et al. (1993) proposed that the presence or absence of macrophytes in shallow systems was the outcome of alternative stable states, driven by complex interactions and feedback loops between the biotic community and abiotic environment. Field evidence suggested that one stable state was associated with the occurrence of macrophytes that provided positive feedback in the form of maintenance of low turbidity and a stable sediment environment. Re-

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sulting high macrophyte productivity and structure favored the maintenance of a diverse and abundant macroinvertebrate community and a fishery dominated by piscivores. Ecosystem-level disturbances such as unexpected lake level fluctuations, introduction of benthic fish communities, or catastrophic storms that caused a stress on macrophyte populations and light conditions often drove these systems toward another equilibrium state because of the loss of the stabilizing influence of macrophytes on light and turbidity. Shallow lakes and littoral regions that had lost macrophyte communities exhibited an alternate stable state characterized by frequent periods of wind-generated sediment resuspension, high turbidity levels, low light penetration, enhanced nutrient recycling, lower macroinvertebrate abundance, benthivorous fish, and the prevalence of nuisance cyanobacteria (James and Barko 1994; Scheffer et al. 1997; Bachmann et al. 2000).

From a management standpoint, it is desirable to promote macrophyte growth to improve water clarity and community structure in shallow systems (Moss et al. 1996). However, it is usually difficult to reestablish macrophyte communities without some form of manipulation to improve light conditions (i.e., reduce resuspension and algal productivity) and to remove destructive benthivorous fish such as common carp (*Cyprinus carpio*). For instance, biomanipulation of shallow Lake Christina (Minnesota) via removal of a fish community dominated by benthivores and restocking with piscivores resulted in greater zooplankton abundance and increased gazing pressure on phytoplankton, reduced bioturbation by benthivores, and increased light penetration that favored macrophyte regrowth and a return to a clear-water state (Hanson and Butler 1994). Another manipulation to improve light conditions for macrophyte growth in lakes that have lost this component is a lake drawdown to promote sediment consolidation and reduction in resuspension potential (i.e., critical shear stress; Scheffer 1998). Fish stocks can also be manipulated during the drawdown to remove bioturbation impacts to the water column. When resuspension is controlled via this lake management technique, successful reestablishment of macrophyte communities can be attained either by planting desirable species or by allowing the native seed bank in the sediments to germinate.

More information is needed regarding the impacts of sediment dewatering and rehydration on sediment N dynamics and N availability for macrophyte growth. Exchangeable sediment ammonium-N (NH_4-N) concentration largely regulates the growth of rooted aquatic macrophytes under conditions of favorable light and temperature (Anderson and Kalff 1986; Barko et al. 1988, 1991). De Groot and Van Wijck (1993) found that rehydration of previously dried river sediment resulted in a pulse of high sediment NH_4-N concentration, presumably because of bacterial cell lysis during the desiccation process. Thus, naturally occurring periods of complete sediment desiccation during drought and water elevation decline in rivers and lakes, or controlled pool elevation drawdowns may result in enhanced concentrations of exchangeable sediment NH_4-N after rehydration, which would favor stimulated macrophyte growth after refill. In contrast, little is known about changes in sediment N concentration during partial sediment dewatering and rehydration. A progressive deepening of the oxic–anoxic interface within the sediment during partial dewatering may result in overall loss of exchangeable sediment NH_4-N via its conversion to nitrate (nitrification) in the oxic layer followed by conversion of nitrate to N_2 gas (denitrification) in the adjacent anoxic layer (Jensen et al. 1994). A loss of exchangeable sediment NH_4-N could induce macrophyte growth limitation. We hypothesized that dewatering of aquatic sediment to different states could alter exchangeable sediment NH_4-N availability for macrophyte growth in addition to affecting the critical shear stress of the sediment. These changes in sediment N chemistry could impact macrophyte reestablishment either positively or negatively in aquatic systems and would, thus, have to be considered in efforts to drive shallow systems toward a clear-water, macrophyte-dominated state. Our overall objectives were to quantify changes in sediment N characteristics and macrophyte growth response on sediments that were experimentally dewatered to lower moisture contents or completely desiccated.

Methods

Experimental sediment dewatering and rehydration

For the first experiment, we quantified changes in sediment physical characteristics and N species as a result of sediment dewatering and rehydration using intact sediment cores collected at 0.75 m depth from a station located in a shallow (mean depth = 0.5 m, maximum depth = 4.4 m, surface area = 610 ha), vegetated backwater region (Lawrence Lake) connected to Pool 8 of the Upper Mississippi River in October 2000. This region was chosen because a pool elevation drawdown was planned for Pool 8 to stimulate shoreline emergent macrophyte growth. The backwater lake is eutrophic (mean summer total N = 1.542 $mg{\cdot}L^{-1}$, total P = 0.179 $mg{\cdot}L^{-1}$, chlorophyll *a* = 35 $mg{\cdot}m^{-3}$) and areas are inhabited by submersed (*Ceratophyllum demersum*, *Potamogeton* sp., *Vallisneria americana*), floating-leaved (*Nelumbo lutea*, *Nuphar lutea* ssp. *variegata*, *Nymphaea odorata*), and emergent (*Scirpus* sp.) macrophytes. Surface sediments (upper 10 cm) at the sampling station exhibited a high moisture content (70–80%), low sediment density (0.2–0.3 $mg{\cdot}L^{-1}$), and an organic matter content of ~11%.

A Wildco KB sediment core sampler (Wildlife Supply Co., Saginaw, Mich.), equipped with a plastic core liner (7.6 cm in diameter, 20 cm in length), was used to collect the sediment cores. In the laboratory, the upper 8.5 cm of sediment was carefully extruded into a core liner that was precut to10 cm. A rubber stopper was used to seal the bottom of each extruded sediment core. The sediment systems were dewatered in the laboratory under controlled temperature conditions (~20 °C) in a darkened room via exposure to ambient air conditions. Sediment cores were dewatered from an initial moisture content of 75% (treatment 1 = 0% dewatered) to a moisture content of 61% (treatment 2 = ~20% dewatered), 32% (treatment 3 = ~60% dewatered), and 5% (treatment 4 = ~95% dewatered). No attempt was made to circulate the air with a fan during the dewatering process. All treatments were run concurrently during the experiment. Thus, cores subjected to treatment 2 were dewatered for 22 days, whereas treatments 3 and 4 required 37 and 62 days of dewatering, respectively. To estimate the loss of moisture from sediment cores over time, the 10-cm core liner and rubber stopper were weighed before and immediately after extrusion of sediment into them. The sediment cores were then weighed every 1–3 days during the dewatering process for estimation of loss of sediment moisture content.

For each treatment, four replicate sediment systems were dewatered to the desired moisture content and sacrificed for analytical determinations in the dewatered state. Three replicate sediment systems for each treatment served as controls and were sealed with rubber stoppers to prevent dewatering. Four additional replicate sediment systems were dewatered to the same desired moisture contents for each treatment but then rehydrated for 2 weeks with filtered (Gelman A/E glass fiber) Lawrence Lake water before sediment analysis. The sediment cores were completely covered with water (100 mL) so that direct rehydration could occur via exposure of the top and sides of the sediment core. The cores were covered with plastic film to minimize evaporative losses during the rehydration period. A 2-week rehydration period was chosen to ensure that the sediments were completely rehydrated before analysis based on a preliminary study (James et al. 2002). The filtered lake water used to rehydrate these cores had an initial concentration of 0.71 mg total soluble $N{\cdot}L^{-1}$, 0.057 mg NH_4-$N{\cdot}L^{-1}$, and 0.60 $mg{\cdot}L^{-1}$ nitrate/nitrite-N (NO_3NO_2-N). The volume of water remaining (i.e., not soaked up by the sediment) after the 2-week rehydration period was measured and analyzed for total N, NH_4-N, and NO_3NO_2-N on a Lachat QuikChem analyzer (Hach Company, Loveland, Co.) using automated analytical techniques (American Public Health Association 1992).

Sediment cores for all treatments were carefully homogenized before physical and chemical analyses. For moisture content and sediment density analyses, a crucible of known volume was completely filled with fresh sediment and dried at 105 °C to a constant weight (Allen et al. 1974). Since sediments were consolidated for treatment 4 (95% dewatered) and did not mold into a volumetric crucible for density analysis, density was determined indirectly by measuring the displacement of distilled water in a volumetric cylinder by a subsample of the fresh sediment and then completely drying that sediment sample at 105 °C. Analysis of total sediment N was performed colorimetrically using Lachat QuikChem procedures after digestion with sulfuric acid, potassium sulfate, and red mercuric oxide (Plumb 1981). Exchangeable sediment N (as NH_4-N) was determined by cation exchange with 1 M sodium chloride according to Bremner (1965).

Rates of N release from sediments under oxic and anoxic conditions were determined using additional sediment cores that were subjected to the same treatments. Cores subjected to dewatering (treatments 2–4) were rehydrated for 2 weeks as described above before determination of rates of N release from the sediments. Immediately after the rehydration period, the cores were drained and transferred to sediment incubation systems (acrylic tubes measuring 6.5 cm in diameter, 25 cm in length) and 300 mL of filtered (Gelman A/E glass fiber) lake water was siphoned onto the sediment to serve as overlying water. The sediment systems were sealed with rubber stoppers, placed in a darkened environmental chamber, and incubated at 20 °C to simulate in situ temperatures during summer. For treatments 3 and 4, consolidation of sediments resulted in a decrease in the sediment surface area and core length. Thus, the sediment surface area was much smaller than the diameter of the incubation system for these treatments. Our goal was to assess N diffusion between the upper surface of the sediment and overlying water for all treatments. To prevent exposure of the side surfaces of the core to overlying water during N release rate analysis, the sides of the core were first gently wrapped in two layers of cellophane (leaving the upper surface exposed) and then fine-grained sand (prewashed in distilled water) was gently packed around the sides of the core after placement in the incubation system.

For each treatment, four replicate sediment systems were incubated for determination of rates of N release under oxic conditions and four additional replicate systems were incubated for determination of rates of N release under anoxic conditions. Control systems that contained only filtered lake water and no sediment were incubated under oxic (three replicates) and anoxic (three replicates) conditions in tandem with the sediment systems for each treatment. The redox environment was controlled in each system by gently bubbling the water with air (for oxic conditions) or N_2 (for anoxic conditions). Samples were collected in the middle of the water column in each system on a daily basis and filtered through a 0.45-μm syringe filter (Gelman GF Acrodisc) for NH_4-N and NO_3NO_2-N analysis (see above). Water removed for analysis was replaced by filtered lake water, preadjusted to the appropriate redox condition, to maintain a constant volume of overlying water in the systems throughout the experiment. Rates of N release from the sediment (milligrams per square metre per day) were calculated as the linear change in mass (corrected for dilution effects of replacement water) in the overlying water divided by time and the upper surface area of the sediment. Changes in the surface area of the sediment core resulting from dewatering were, therefore, factored into the rate calculations.

Macrophyte growth response

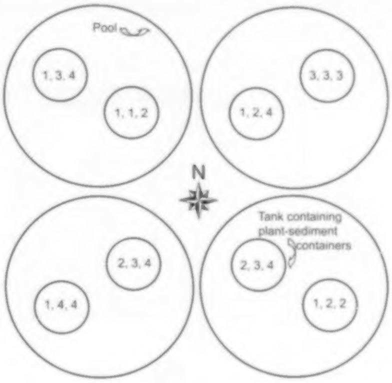
For the second experiment, sediments collected from the same location in Lawrence Lake at the same time in October 2000 were used to examine macrophyte growth response as a function of sediment dewatering and rehydration. Larger, 4-L containers (16 cm wide, 16 cm deep, 22 cm high) filled with 3 kg of completely homogenized (gently stirred) sediments were used for the macrophyte growth response experiment to accommodate root development. The initial depth of the sediment in the plant containers was 10 cm. A preliminary study was conducted to determine the rate of dewatering (percent moisture lost per day) to predict the length of time required to dewater sediments to specific moisture contents. The containers filled with sediment were sealed and refrigerated at 4 °C until the start of the dewatering process. Using the dewatering rate information collected in the preliminary study, containers were pulled from refrigeration at various time intervals, opened, and allowed to dry to the target moisture contents (i.e., 20%, 60%, and 95% dewatered) at room temperature (~20 °C) in a darkened area. The objective was to have all sediment containers reach their target moisture contents simultaneously by early June 2001. All sediment containers were rehydrated with 1 L of water from a local tap water source (Ca = 57 $mg \cdot L^{-1}$, conductivity = 422 μS, Mg = 28 $mg \cdot L^{-1}$, NO_2NO_3-N = 0.2 $mg \cdot L^{-1}$, K = 0.8 $mg \cdot L^{-1}$, Na = 1.6 $mg \cdot L^{-1}$, SO_4 = 21 $mg \cdot L^{-1}$, pH = 7.8) for a period of 4 days before planting in June. Prewashed fine-grained sand was poured along the sides of compacted (i.e., treatments 3 and 4), rehydrated sediment to fill in lost sediment volume before planting. However, cellophane was not used to separate the sediment from the sand so that root development would not be impeded.

Potamogeton pectinatus was used as the experimental macrophyte in the growth response studies. Propagules, obtained commercially (Kester's W.F.G. Nurseries, Omro, Wis.), were germinated before initiation of the experiment and transplanted to the sediment containers immediately after rehydration of the dewatered sediments. Six replicates were planted for each treatment for a total of 24 sediment containers. One sprout was planted in each sediment container. Additional sediment containers (six replicates per treatment × four treatments), without macrophytes, served as controls to monitor initial sediment conditions (moisture content, sediment density, exchangeable sediment NH_4-N; see above for a description of analytical methods) before macrophyte growth.

The planted sediment containers were incubated in clear plastic tanks (1.2 m in diameter, 1.2 m high) that were placed in a large outdoor facility consisting of four swimming pools (4.6 m in diameter, 1.2 m deep). Two tanks were placed in each pool (Fig. 1). The swimming pools served as water baths to moderate temperatures in the summer. The temperature in each pool was controlled to a certain extent with water temperature controllers (Remcor CFF500). Water clarity in the pools was maintained using sand filters. Only three planted sediment containers were placed at the bottom of each tank (1 m from the water surface) to minimize crowding and shading during the growing period. Placement of the planted sediment containers in tanks in the outdoor facility was randomly determined. All tanks and pools were filled with tap water before the start of the experiment.

During the growing period, the tanks were protected with clear acrylic covers that allowed airflow from the sides but prevented rain and debris from falling into the tanks. Airlift pumps were placed in each tank to promote circulation. At weekly intervals, half of the water in each tank was carefully flushed with replacement fresh tap water. Each tank was also filtered one or two times a week (diatomaceous earth filters) to minimize and remove algal growth. The experiment was initiated on 18 June and ended on 31 July 2001. At the end of the experiment, shoots were harvested from each container for determination of biomass and N content. Biomass was measured to the nearest 0.1 g after drying at 40 °C. Macrophyte N content was determined according to Allen et al. (1974).

Fig. 1. Schematic diagram of experimental pools and tanks. Sediment containers planted with *P. pectinatus* were randomly assigned to different tanks for statistical analyses. 1 = 0% dewatered sediments, 2 = 20% dewatered sediments, 3 = 60% dewatered sediments, and 4 = 95% dewatered sediments.



Statistical analyses

Statistical analyses were performed using the software program Statistical Analysis System (SAS Institute Inc. 1994). For each experiment, the Shapiro–Wilk statistic was used for normality testing. Analysis of significant variations between and among treatments was performed using analysis of variance (ANOVA) (n = 24) (Waller–Duncan) (SAS Institute Inc. 1994) after natural logarithmic transformation of the data. Replication of treatments used for ANOVA in the first experiment for analysis of moisture content, sediment density, total sediment N, and exchangeable sediment NH_4-N was three for the sealed controls, four for sediment cores in the dewatered state, and four for sediment cores in the rehydrated state. For the determination of NH_4-N and NO_3NO_2-N release from sediments, four replicates were incubated under oxic and anoxic conditions for each treatment. For the macrophyte growth experiment, six replicates were used in each treatment. Regression analysis (n = 24) (SAS Institute Inc. 1994) was used for the macrophyte growth experiment to examine best fit between macrophyte biomass and exchangeable sediment NH_4-N, sediment moisture content, and sediment density.

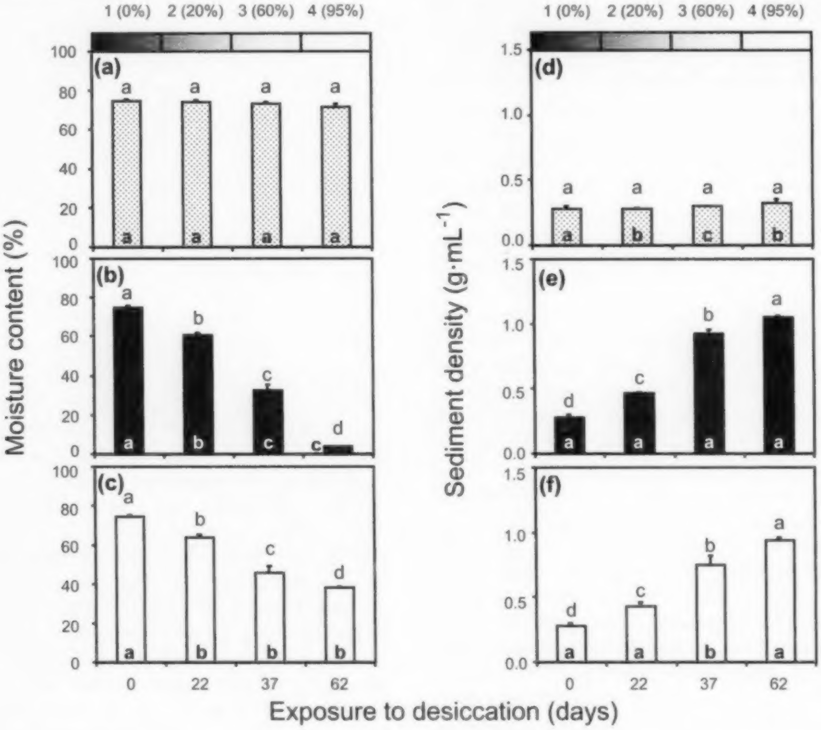
Results

Experimental sediment dewatering and rehydration

During the dewatering process in experiment 1, sediment cores lost moisture at a rate of 1.5% water mass per day. Near complete dryness occurred in ~60 days. Sediment cores consolidated as a consequence of water loss, resulting in a decline in sediment core length from an initial mean of 8.5 cm to a final mean of 4.6 cm after nearly complete desiccation. Sediment core diameter declined from an initial mean of 6.6 cm to a final mean of 4.5 cm after nearly complete desiccation.

Mean moisture content and sediment density exhibited a hysteretic pattern as a result of dewatering and rehydration (Fig. 2). Rehydration of sediments that were dewatered by 20%, 60%, and 95% resulted in an overall lower mean moisture content (ANOVA; df = 15, F = 60.69, $p < 0.01$), and higher mean density (ANOVA; df = 15, F = 62.88, $p < 0.01$), compared with initial mean sediment conditions (i.e., day 0 and no dewatering). As the percentage of water re-

Fig. 2. Variations in mean (*a–c*) moisture content and (*d–f*) sediment density as a function of exposure of intact sediment cores to desiccation (experiment 1). Stippled bars represent means for sediment core control systems, which were not exposed to desiccation, solid bars represent means for sediment core systems in the dewatered state, and open bars represent means for sediment core systems after dewatering and rehydration. Vertical lines above the bars represent 1 SE. Different letters above the bars represent significant differences as a function of exposure to desiccation (i.e., between-treatment effects), whereas letters inside the bars represent significant differences as a function of the sediment core state for each time period (i.e., among-treatment effects for control, dewatered, or dewatered and rehydrated sediment cores).

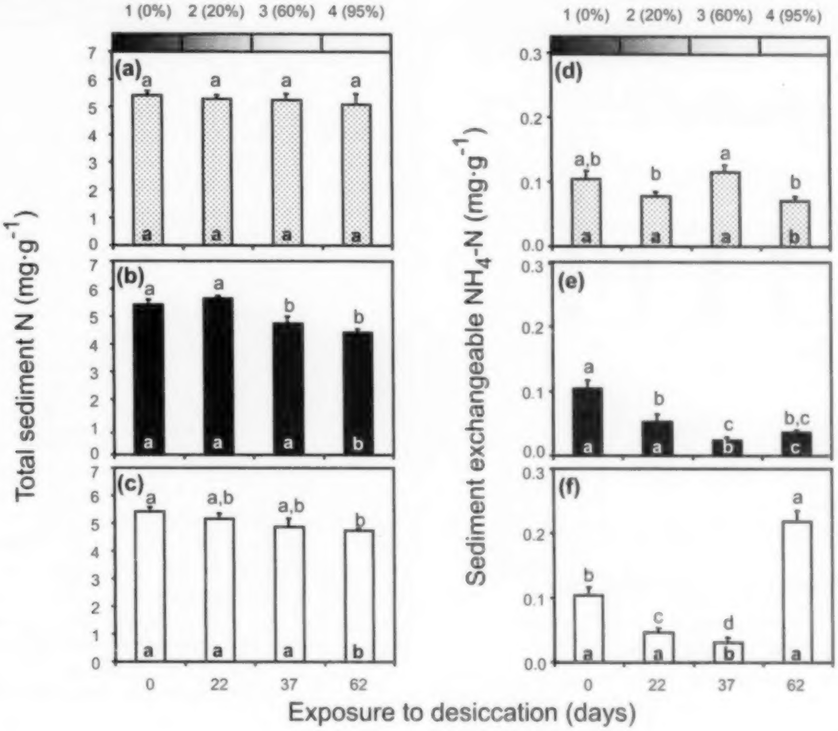


moved from sediment increased, there was a trend of greater rebound in mean moisture content and mean density conditions upon rehydration. Thus, drier sediment gained more water than wetter sediment during the rehydration process. However, the mean moisture content of rehydrated sediment was lower, whereas mean sediment density was higher, as a function of increased dewatering. In contrast, mean moisture content and sediment density in the control treatments, which were not dewatered, remained similar to initial mean sediment conditions over time.

The mean concentration of exchangeable sediment NH_4-N declined significantly (ANOVA; df = 15, $F = 32.67$, $p < 0.01$) compared with initial concentrations as a function of the percentage of water removed (Fig. 3). For sediments that were dewatered by 20% and 60%, rehydration did not result in any additional changes in mean concentration, as exchangeable sediment NH_4-N remained low relative to initial conditions for these treatments. However, sediments dewatered by 95% exhibited substantial increases in mean exchangeable sediment NH_4-N after rehydration. In contrast, concentrations of exchangeable sediment NH_4-N in control sediments fluctuated over time around a grand mean of 0.09 mg·L^{-1} and exhibited no clear trend over time. Total sediment N remained constant over time for control sediments but exhibited a trend of significantly lower concentrations compared with initial conditions for sediment dewatered by 60% and 95% and for sediment dewatered by 95% and rehydrated (ANOVA; df = 15, $F = 2.7$, $p < 0.09$) (Fig. 3). Overall, 95% dewatering of sediment resulted in a net loss of nearly 18% of the total sediment N. Changes in the concentration of total N and NH_4-N in the water used to rehydrate these sediments accounted for less than 2% of the loss in total sediment N.

Similar to patterns observed for mean exchangeable sediment NH_4-N, mean rates of NH_4-N release from sediments declined substantially under both oxic (ANOVA; df = 15, $F = 4028.38$, $p < 0.01$) and anoxic conditions (ANOVA; df = 13, $F = 517.27$, $p < 0.01$) as a result of dewatering sediment by 20% and 60% (Fig. 4). In particular, mean rates of NH_4-N release from sediments were undetectable under oxic conditions for sediments dewatered by 60%. Dewatering the sediment by 95%, followed by rehydration, resulted in a marked increase in the mean rate of NH_4-N release from sediments compared with initial rates. Mean rates of NO_3NO_2-N release from sediments under oxic conditions exhibited a similar pattern of declining values when sediments were partially dewatered and then rehydrated and a very high rate due to nearly complete desiccation and rehydration (ANOVA; df =

Fig. 3. Variations in mean (*a–c*) total sediment N and (*d–f*) exchangeable sediment NH_4-N as a function of exposure of intact sediment cores to desiccation (experiment 1). Stippled bars represent means for sediment core control systems, which were not exposed to desiccation, solid bars represent means for sediment core systems in the dewatered state, and open bars represent means for sediment core systems after dewatering and rehydration. Vertical lines above the bars represent 1 SE. Different letters above the bars represent significant differences as a function of exposure to desiccation (i.e., between-treatment effects), whereas letters inside the bars represent significant differences as a function of the sediment core state for each time period (i.e., among-treatment effects for control, dewatered, or dewatered and rehydrated sediment cores).



14; F value = 62.54; $p < 0.01$). We did not detect fluxes of NO_3NO_2-N from sediments under anoxic conditions for any treatment.

Macrophyte growth response

In the second experiment, *P. pectinatus* grown on sediments that were dewatered by 20% and 60% exhibited a trend of lower mean biomass (ANOVA; df = 23, $F = 22.16$, $p < 0.01$) and macrophyte N mass (ANOVA; df = 23, $F = 7.66$, $p < 0.01$) versus growth on sediment that was not dewatered (Fig. 5). Mean biomass and macrophyte N mass were significantly higher for macrophytes grown on sediment that was dewatered by 95% compared with biomass indicators for macrophytes grown on partially dewatered sediments (i.e., 20% and 60%). Mean macrophyte N mass was also significantly higher for macrophytes grown on sediment that was dewatered by 95% versus macrophytes grown on sediment that was not dewatered. However, clear statistical differences were not observed between these two treatments for mean biomass. Regression analysis indicated that most of the variation in macrophyte biomass and N content was attributed to variations in exchangeable sediment NH_4-N ($n = 24$, $r^2 = 0.64$ and 0.57 for biomass and macrophyte N mass, respectively) versus sediment moisture content and density.

Discussion

Exchangeable sediment NH_4-N levels could be experimentally manipulated to a certain extent via dewatering and rehydration to regulate the growth of an experimental macrophyte, *P. pectinatus*. Partial dewatering (i.e., by 20% and 60%) of sediments followed by rehydration resulted in a decrease in mean exchangeable sediment NH_4-N concentrations and a corresponding depression in the growth of *P. pectinatus* relative to macrophytes grown on sediments that were not dewatered. Conversely, 95% dewatering of sediment followed by rehydration resulted in a substantial increase in exchangeable sediment NH_4-N concentration and a corresponding increase in the growth of *P. pectinatus* relative to biomass levels for those macrophytes grown on partially dewatered sediments. Although growth was robust and mean biomass levels were greatest for treatment 4 compared with other treatments, we did not observe statistically significant differences in mean biomass for macrophytes grown on sediments that were not dewatered versus sediments that

Fig. 4. Variations in mean rates of (*a*) NH_4-N and (*b*) NO_3NO_2-N release from sediments under oxic and anoxic conditions as a function of exposure of intact sediment cores to desiccation (experiment 1). Open bars represent rates under oxic conditions and solid bars represent rates under anoxic conditions (note the logarithmic scale on the *y*-axis). Rates of NO_3NO_2-N release from sediments under anoxic conditions were zero over all exposure times. Vertical lines above bars represent 1 SE. Different letters above the bars represent significant differences as a function of exposure to desiccation (i.e., between-treatment effects).

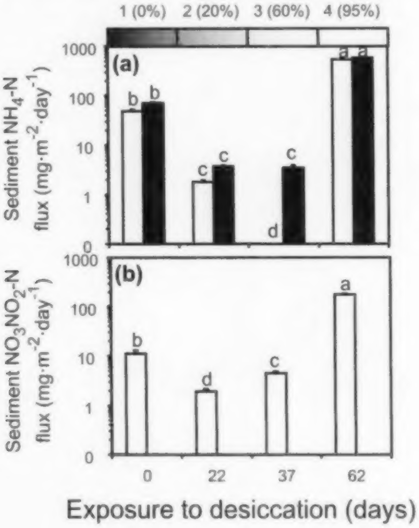
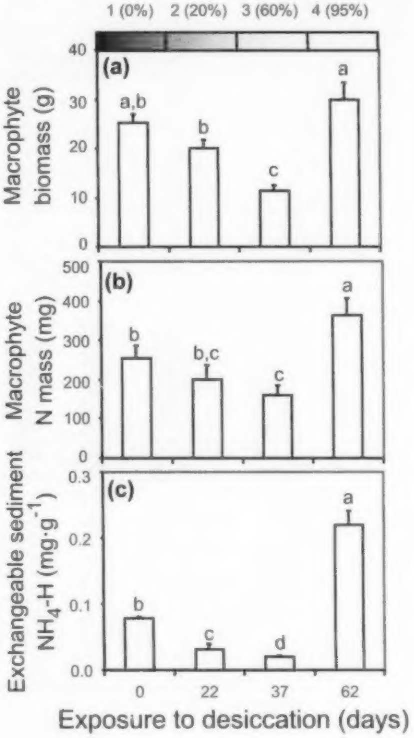


Fig. 5. Variations in mean (*a*) macrophyte biomass, (*b*) macrophyte N mass, and (*c*) exchangeable sediment NH_4-N of planted sediment containers as a function of exposure to dessication (experiment 2). Vertical lines above bars represent 1 SE. Different letters above the bars represent significant differences as a function of exposure to desiccation (i.e., between-treatment effects).



were dewatered by 95%. Perhaps the lack of a greater growth response for macrophytes grown on sediments that were dewatered by 95% was due to the very high sediment density, even after rehydration, which could have impeded root development and deep penetration into the sediment, thereby limiting the maximum potential growth that might be achieved on these N-rich sediments. Unlike our experimental setting, complete desiccation and consolidation of the entire rooting depth medium (0.5 m or greater) is unlikely to occur under most in situ conditions. Nevertheless, variations in exchangeable sediment NH_4-N incurred as a result of sediment dewatering accounted for up to 64% of the variation in mean macrophyte biomass, suggesting linkages between the nutritional quality of the sediment and macrophyte growth.

Processes driving changes in the exchangeable sediment NH_4-N pool during sediment dewatering and rehydration are not entirely known. A reduction in exchangeable sediment NH_4-N concentration for partially dewatered sediments (i.e., sediments dewatered by 20% and 60%) may have been associated with the occurrence of nitrification coupled with denitrification (Mitchell and Baldwin 1999) at the oxic–anoxic interface within the sediment. The oxygenated microzone may have moved further inward toward the interior of the core as the sediment dried, providing favorable conditions for bacterial nitrification of exchangeable sediment NH_4-N located deeper within the core that was not previously exposed to oxygenated conditions under initial conditions, resulting in its loss via conversion to NO_3. Nitrate byproducts diffusing into the anoxic zone of the core could then be converted to N_2 gas via bacterial denitrification (Jensen et al. 1994). An overall reduction in sediment total N concentrations (18% reduction over sediments that were not dewatered) as a result of dewatering sediment further suggested the likelihood that exchangeable sediment NH_4-N was depleted because of coupled nitrification and denitrification during the sediment dewatering process. Our observations of total sediment N loss because of sediment dewatering are consistent with the findings of De Groot and Van Wijck (1993) and James et al. (2001) for dewatered marsh and lake sediments, respectively. Others have demonstrated the loss of total N from dewatered and rehydrated soils (Reddy and Patrick 1975; Minzoni et al. 1988), suggesting denitrification.

In contrast, nearly complete dewatering (i.e., 95%) followed by rehydration resulted in very high exchangeable sediment NH_4-N concentrations. Others have found high porewater NH_4-N and exchangeable sediment NH_4-N after complete dewatering and rehydration of sediments (De Groot and Van Wijck 1993; James et al. 2001). One mechanism believed to be responsible for this response is high microbial mortality resulting from desiccation and release of soluble N during cell lysis (De Groot and Van Wijck 1993; Baldwin and Mitchell 2000). However, rehydration of dewatered sediments with initially oxygenated lake water may have also accelerated mineralization of organic N to NH_4-N by newly populated microbial communities, additionally contributing to the high concentrations of exchangeable sediment NH_4-N.

Our research suggested that sediment dewatering via exposure to the air during natural drought conditions or operationally controlled pool drawdowns could alter exchangeable sediment NH_4-N concentrations negatively or positively, depending on the extent of dewatering, thereby affecting macrophyte growth and total sediment N concentrations. These changes need to be considered when evaluating drawdown as a technique for shifting shallow systems to a macrophyte-dominated state. Changes incurred in sediments because of dewatering and desiccation also have implications for N cycling and fate in aquatic systems. For instance, drought-driven exposure and partial dewatering of N-rich sediments in lakes and rivers may force temporary N limitation of macrophyte growth on these substrates during subsequent seasons of refill. Prolonged exposure and complete desiccation of sediments may stimulate release of NH_4-N, which could enhance macrophyte productivity under N-limiting conditions. For aquatic ecosystem management purposes, it might be desirable to partially dewater littoral sediment by a controlled pool drawdown to reduce exchangeable sediment NH_4-N availability for macrophyte growth for control of N-demanding species such as *Myriophyllum spicatum*. Conversely, complete sediment dewatering and desiccation might be desirable to temporarily enhance exchangeable sediment NH_4-N availability for macrophyte growth. The latter scenario might be applicable for encouragement of macrophyte growth in shallow aquatic systems that have lost this component and contain N-poor sediments. In contrast, mobilization of sediment NH_4-N via complete sediment desiccation might not be desirable for N-enriched systems. Findings from this study may also have applications in the development of strategies for mitigating high NH_4-N concentrations in the sediment or inducing overall sediment N dissipation in N-rich systems such as the Mississippi River. More information is needed regarding processes driving N transformations and losses in the sediment as a result of dewatering (i.e., nitrification–denitrification in relation to changes in the oxic–anoxic boundary in the sediment) over a range of different sediment types and sediment N concentrations to better understand N dynamics in aquatic systems and to improve applications for shifting shallow lakes toward more desirable clear-water states via sediment dewatering.

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Effects of nonrandom mating schemes to delay the inbreeding accumulation in cultured populations of coho salmon (*Oncorhynchus kisutch*)

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Abstract: Chilean salmon culture is based on a high degree of artificial selection, which has had the tendency to increase the inbreeding (F). Three types of nonrandom mating were evaluated to control the inbreeding in two best linear unbiased prediction selected coho salmon (*Oncorhynchus kisutch*) populations (even and odd). These included compensatory mating on the basis of breeding values (C), modified compensatory mating (C1) based on the family mean of breeding values, and mating that minimized the mean co-ancestry of the group selected (MC scheme). In the odd population, the MC scheme (F = 2.0%) reduced the increase in inbreeding of the next generation by 50% and 46% when compared with random mating of selected individuals with sib mating restricted (F = 3.9%) and with C (F = 3.7%), respectively. In the even population, the MC scheme reduced the increase in inbreeding by 14% compared with C1 (9.7 versus 11.2). In both populations, the MC scheme also reduced variance in inbreeding (even, 59%; odd, 39%). Thus, the MC scheme was more efficient in reducing the increase and variance of inbreeding, thus limiting the expression of inbreeding depression. Although the MC scheme was more time consuming, we recommend this scheme to carry out crosses in each generation.

Résumé : La culture du saumon au Chili comporte une forte composante de sélection artificielle, ce qui a eu tendance à augmenter la consanguinité (F). Nous avons évalué trois types de croisements non aléatoires dans le but de contrôler la consanguinité dans deux populations de saumons coho (*Oncorhynchus kisutch*) sélectionnées par la méthode de la meilleure prédiction linéaire non biaisée (paire et impaire). Les trois types comprenaient le croisement compensatoire basé sur la valeur génétique (C), le croisement compensatoire modifié (C1) basé sur la valeur génétique moyenne de la famille et un croisement qui minimise la filiation commune moyenne du groupe sélectionné (MC). Chez la population impaire, le type MC (F = 2,0 %) réduit l'augmentation de la consanguinité de la génération suivante respectivement de 50 % et de 46 % par comparaison au croisement aléatoire d'individus sélectionnés (avec restriction de l'accouplement entre membres d'une même famille) (F = 3,9 %) et au croisement de type C (F = 3,7 %). Dans la population paire, le croisement de type MC réduit l'accroissement de la consanguinité de 14 % par rapport au croisement de type C1 (9,7 % plutôt que 11,2 %). Chez les deux populations, le croisement de type MC réduit aussi la variance de la consanguinité (paire, 59 %; impaire, 39 %). Le croisement de type MC est donc plus efficace pour réduire l'augmentation et la variance de la consanguinité et il diminue ainsi l'expression de la dépression de consanguinité. Bien que les croisements de type MC requièrent plus de temps, nous les recommandons pour effectuer les croisements dans toutes les générations.

[Traduit par la Rédaction]

Introduction

Genetic improvement of fishes in aquaculture has recently benefited by incorporation of the "best linear unbiased predictor" (BLUP) (Henderson 1975) for breeding values when using the animal model for genetic evaluation (Gjoen and Gjerde 1998; Martínez et al. 1999). This procedure maximizes the correlation between the true and the predicted breeding values using information from all parents in the present generation and of preceding generations, as well as the individual's own data. This animal estimation produces a more exact estimation for the breeding value and increases the genetic response compared with other methods, e.g., phenotypic selection (Belonsky and Kennedy 1988). However, given that the animal model favours selection of related individuals, it also produces an increase in the rate of inbreeding and a long-term reduction in the genetic variance (Belonsky and Kennedy 1988; Quinton et al. 1992). There

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have been few empirical evaluations of the rate of inbreeding in fish populations subject to artificial selection. In rainbow trout (*Oncorhynchus mykiss*) populations, Su et al. (1996) estimated a rate of inbreeding of 1.3–1.7% per generation, whereas Pante et al. (2001*a*) for other populations found rates of inbreeding of 0.53–2% per generation. J.A. Gallardo et al. (unpublished data) recently found that two populations of coho salmon (*Oncorhynchus kisutch*) selected following an animal model gave inbreeding rates of between 1.05 and 2.37% per generation.

Limiting inbreeding in selection programs is important, as higher levels of inbreeding increase the probability of inbreeding depression. Estimation of inbreeding depression in fishes has consistently shown that consanguineous progeny have lower viability and less growth and also show lower resistance to infection by protozoans (Su et al. 1996; Pante et al. 2001*b*; Arkush et al. 2002).

Various methods have been devised to reduce tendencies towards an increase in the rate of inbreeding produced by artificial selection (i.e., BLUP or mass selection) while at the same time maintaining a high genetic gain (Villanueva et al. 1996; Meuwissen 1997; Wu and Schaeffer 2000). These include (*i*) BLUP estimations of breeding values with incremented heritabilities (Grundy et al. 1994; Wu and Schaeffer 2000), (*ii*) estimations using a suboptimal selection criterion, for example, giving a lower weighting to family information (Verrier et al. 1993; Wu and Schaeffer 2000), (*iii*) selection methods that manipulate the genetic contribution to the next generation of each candidate selected (Toro and Nieto 1984; Meuwissen 1997), (*d*) nonrandom mating schemes such as compensatory mating or those that minimize the average co-ancestry of the group selected (Toro and Perez-Enciso 1990; Caballero et al. 1996), and (*e*) mixed methods that used some preceding approaches simultaneously (Grundy et al. 1994; Sonesson and Meuwissen 2000). Regardless of which method is to be applied for selecting the reproducers for the next generation, the mating scheme among selected candidates is a determining factor in reducing the inbreeding rate, with random mating of selected individuals the least efficient (Grundy et al. 1994; Caballero et al. 1996; Sonesson and Meuwissen 2000). Practical considerations and reproductive restrictions may also limit the efficiency of these methods in reducing the rate of inbreeding (Meuwissen 1997; Fernández and Toro 1999). To date, few studies have evaluated the methods cited above (Sanchez et al. 1999; Weigel and Lin 2000), and none has been evaluated taking into account the characteristics of programs used in genetic selection in fishes.

The objective of the present study was to evaluate the effects of different nonrandom mating schemes in reducing inbreeding accumulation over a short term (one generation) in two populations of coho salmon selected by means of an animal model. This evaluation takes into account a series of practical and reproductive limitations relevant to various artificial selection programs in salmonids.

Materials and methods

Study populations

The study was based on data from two coho salmon populations belonging to a genetic improvement center (Centro de mejoramiento genético (CMG)) maintained by the Institute for Fisheries Development and the University of Chile in Coyhaique (XI Region, Chile). The two populations, termed "even" and "odd", were produced in 1992 and 1993, respectively, and are managed in a 2-year productive cycle. Each year class was produced using a hierarchical mating system by means of mating of 30–35 males with approximately 100 females, producing a population of 30–35 families of half-siblings (HS) and 100 families of full siblings (FS). Both populations were submitted to artificial selection: five generations for the even population and four generations for the odd population. Characters selected included the weight at harvest using the BLUP prediction of the breeding value and early spawning by means of phenotypic selection. More details on characters and origins of the populations are given in Martínez et al. (1999) and Winkler et al. (1999). The genealogy has been recorded from the beginning of the program to the present, giving calculated mean rates of inbreeding of 2.37% for the even population and 1.05% for the odd population. The mean inbreeding calculated in the previous year class investigated in this study was 3.16 ± 2.65% (year class 1999) and 9.4 ± 2.7% (year class 2000). The inbreeding rate was greater in the even population than in the odd population as a direct consequence of the smaller number of founder individuals in the former population ($N_e = 61$ and 106, respectively).

Mating schemes

The following mating schemes were compared in terms of the level of predicted next-generation inbreeding. (*i*) For random mating of selected individuals (R), crosses between selected males and females were carried out randomly, avoiding crosses between HS and FS. This scheme was routinely used at the CMG until 2001. (*ii*) In compensatory mating (C), a ranking was obtained following the breeding values for harvest weight of all individuals, grouped per spawning day. Based on this ranking, a mating system was simulated in which the male with the highest breeding value was assigned to cross with the female with the lowest breeding value, followed by assignment of the second-ranked male with the next-to-last ranked female and so forth until all individuals had been assigned. If the female was HS or FS with the assigned male, the assignment was replaced with a female that had the next highest breeding value, assigning the next male to this female. (*iii*) For modified compensatory mating (C1), a ranking was arranged of breeding values for harvest weight based on the average of paternal HS. A total of 19 families of half-brothers and 34 families of half-sisters were separately classified. The number of individuals per HS family varied between 1 and 59 for the males and between 3 and 86 for the females. Compensatory matings were carried out in a similar manner to the preceding scheme based on the family average. The male or female breeder with the highest family breeding value was crossed with the female or male with the lowest value for a particular spawning day and so forth. Crosses between HS and FS were avoided. (*iv*) The minimum co-ancestry (MC) scheme took into account the use of all genealogical information to assign the crosses and consisted of selecting a group for crosses of males and females having the minimum average co-ancestry. Co-ancestry between two individuals is the

Table 1. Number of sires (S), dams (D), and crosses (S × D) evaluated per spawning day in two year classes of coho salmon.

2001 year class				2002 year class			
Spawning day	S	D	S × D	Spawning day	S	D	S × D
26 April	8	12	96	27 April	6	12	72
27 April	11	22	242	29 April	5	9	45
30 April	15	28	420	3 May	17	30	510
1 May	17	24	408	7 May	17	29	493
4 May	13	25	325	7 May[a]	14	28	392
8 May	20	40	800	9 May	15	33	495
9 May	24	38	912	9 May[a]	7	13	91
12 May	28	47	1316	11 May	10	18	180
16 May	14	21	294	14 May	16	24	384
22 May	8	12	96	17 May	8	9	72
				20 May	7	8	56
				24 May	5	9	45

[a] Crosses carried out in the afternoon were considered independent from crosses carried out in the morning.

same as the probability that two alleles for a locus, each one taken from two different individuals, would be equal through descendency (Malecot 1948). This is equivalent to the inbreeding of the hypothetical progeny of these individuals if they are crossed (Falconer and Mackay 1996). Thus, minimization of the co-ancestry of a group of breeders is equivalent to reducing the inbreeding of their progeny. Co-ancestry coefficients were determined using PEDIGREE VIEWER program version 4.9 (B. Kinghorn and S. Kinghorn, University of New England, Armidale, New South Wales, Australia). Determination of which group of crosses minimizes the average co-ancestry has been generally treated as a problem in linear programming (Jansen and Wilton 1985; Sanchez et al. 1999; Weigel and Lin 2000), and this is the strategy used in this study. The following objective function was minimized in the present study:

$$(1) \qquad \frac{\sum\sum x_{ij}(f_{ij})}{H}$$

This equation represents the average co-ancestry of the group selected, where x_{ij} is a decision variable that indicates whether the sire and dam are paired ($x_{ij} = 1$) or not ($x_{ij} = 0$), f_{ij} is the coefficient of co-ancestry between the sire i and the dam j, and H is the number of females spawned on a given day (usually between 10 and 40). The objective function must comply with the following conditions:

$$(2) \qquad \Sigma\Sigma x_{ij} = H$$

$$(3) \qquad \Sigma\Sigma x_{ij} = 1 \qquad \text{(per each mother)}$$

$$(4) \qquad \Sigma\Sigma x_{ij} = 1, 2, 3 \quad \text{(per each father)}$$

Equation 2 indicates the number of crosses carried out for a given day, eq. 3 indicates that one dam was crossed with only one sire, and eq. 4 indicates that one sire could cross with one, two, or three females on a particular day. The vector of crosses was obtained using the Hiper Lindo program (Lindo Systems, Inc. 1985).

Practical and reproductive restrictions

The two main limitations in this study were related to the number of crosses that could be evaluated simultaneously. The number of crosses evaluated was limited by the number of breeders successfully spawned each day (Table 1) and not by the entire group of breeders selected for the animal model. This resulted in 58 sires and 279 dams in 2001 (15 660 possible crosses) and 55 sires and 266 dams in 2002 (14 630 possible crosses). Management of the spawning at the CMG was carried out following natural maturation in the fishes, which occurred in periods of 5–6 weeks, following a normal distribution. Gametes were obtained day by day from mature breeders, and after the quantity and quality of the gametes were determined, it was possible to obtain "optimum" crosses using methods cited above for each particular day. In relation to the preceding, a practical limitation was also included as follows. Because of limitations on personnel and space at the CMG, two groups of mature fishes were spawned separately some days, one in the morning and the other in the afternoon. Crosses of each group were optimized separately in 2002.

Evaluation of the mating schemes

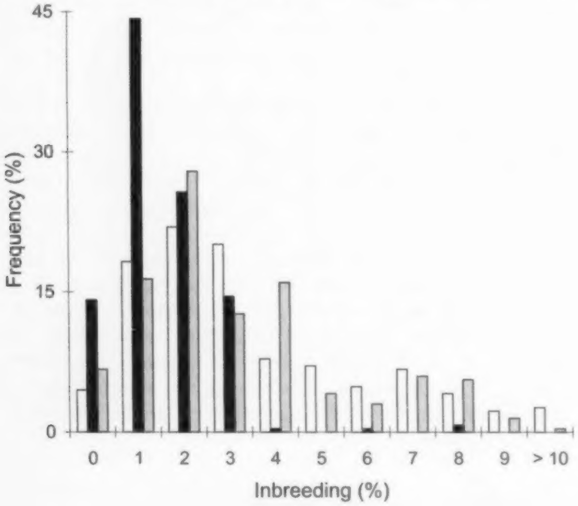
Evaluation of the different mating schemes was carried out in two steps, each one involving the year class of each population (2001–2002). Only the reproductive limitation was applied to year class 2001. Here, the efficiency in reducing inbreeding in the next generation was compared between two nonrandom mating schemes, including the C mating and MC mating versus the R mating schemes of selected individuals carried out in this year. In year class 2002, the CMG placed into practice the C1 mating scheme instead of the R mating schemes used until this time. In this stage, both reproductive and practical restrictions were taken into account. The efficiency of the C1 scheme was compared with that of the MC scheme, which had been found to be the better scheme from the previous stage. Following the system of hierarchical crosses used in this program, and with the objective that the comparisons between the different schemes should be carried out under the same expected genetic response level, each breeder was utilized the same number of times in

Table 2. Mean inbreeding (*F*) and inbreeding ratios between random mating of selected individuals (R), minimum co-ancestry (MC), and compensatory (C) mating of the coho salmon 2001 year class by spawning day.

	Mean *F*			Inbreeding ratio					
Spawning day	R	MC	C	MC/R	*P*	MC/C	*P*	C/R	*P*
26 April	4.77	1.92	5.16	0.40	*	0.37	*	1.08	ns
27 April	3.04	1.81	3.07	0.60	**	0.59	ns	1.01	ns
30 April	3.48	1.76	3.47	0.51	**	0.51	**	1.00	ns
1 May	4.57	1.99	3.33	0.44	**	0.60	**	0.73	ns
4 May	3.67	1.91	2.95	0.52	*	0.65	**	0.80	ns
8 May	4.21	1.91	3.47	0.45	**	0.55	**	0.82	ns
9 May	3.86	1.94	3.19	0.50	**	0.61	**	0.83	ns
12 May	4.13	1.82	4.17	0.44	**	0.44	**	1.01	ns
16 May	4.15	2.61	4.20	0.63	*	0.62	**	1.01	ns
22 May	4.57	3.65	5.91	0.80	ns	0.62	ns	1.29	ns
Mean	3.99	2.01	3.70	0.50	***	0.54	***	0.93	ns

Note: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, nonsignificant.

Fig. 1. Frequency distribution of inbreeding in hypothetical progeny of the coho salmon 2001 year class for random mating of selected individuals (open bars), compensatory mating (shaded bars), and minimum co-ancestry mating (solid bars) ($N = 269$ families).



each mating scheme (identical genetic contribution of each parent between mating schemes).

Statistical analyses

In both year classes, the efficacy of each scheme in reducing the inbreeding rate was evaluated by day and by spawning season, at the population level. The total number of crosses evaluated per spawning day was between 96 and 1316 for the 2001 year class and between 45 and 510 for the 2002 year class (Table 1). To compare mean inbreeding per spawning day among schemes, a nonparametric Mann–Whitney test was used, whereas the variances of inbreeding were compared using an *F* test (Zar 1996). A Kolmogorov–Smirnov analysis (Zar 1996) was used at the population level to compare the distribution of frequencies of inbreeding arising from all of the crosses selected in each year class by the mating schemes.

Results

Evaluation of mating schemes for the 2001 year class

The MC scheme was more efficient in reducing the average inbreeding and the variance of the inbreeding in the next generation in comparison with the other mating schemes evaluated. The MC scheme reduced the inbreeding ($P < 0.001$) 50% compared with the R mating schemes of selected individuals and 46% compared with the C mating scheme (Table 2). In relation to spawning day, this reduction in average inbreeding ranged between 20% and 60%. In comparison, the C scheme was only slightly more effective (7%) at reducing inbreeding in comparison with the R scheme, although none of the daily differences in the tests was significant (Table 2). Also, the MC scheme significantly reduced ($P < 0.001$) the variance in the inbreeding by 59% compared with the R scheme and 54% compared with the C scheme (Fig. 1). Conversely, the C scheme reduced ($P < 0.05$) the variance of the inbreeding only 12% when compared with the R scheme (Fig. 1). Consequently, the frequency distribution of inbreeding was significantly different between MC and R (Kolmogorov–Smirnov test, $D = 0.42$, $P < 0.001$) and between MC and C (Kolmogorov–Smirnov test, $D = 0.37$, $P < 0.001$) but not between C and R (Kolmogorov–Smirnov test, $D = 0.081$, *P* not significant).

Evaluation of mating schemes for the 2002 year class

The MC scheme was more efficient in reducing the inbreeding in all of the parameters evaluated in comparison with the C1 scheme. The MC scheme reduced the inbreeding by an average of 14% in comparison with C1 (Table 3), whereas on a per spawning day basis, this reduction ranged between 5% and 17%. As with year class 2001, the MC scheme also significantly reduced ($P < 0.001$) the variance of the inbreeding, with an average reduction of 39% (Table 3). Consequently, the frequency distribution of the inbreeding was significantly different (Kolmogorov–Smirnov test, $D = 0.33$, $P < 0.001$) between the two nonrandom mating schemes (data not shown).

The practical and reproductive restrictions evaluated in this year class limited the efficiency of the MC scheme over the C1 scheme in inbreeding reduction when less than

Table 3. Mean inbreeding (*F*) and standard deviation of the inbreeding and the inbreeding ratio between minimum co-ascendancy (MC) and modified compensatory (C1) mating in the coho salmon 2002 year class per number of crosses evaluated (*N*).

	Mean *F*				Standard deviation			
			Inbreeding ratio				Inbreeding ratio	
N	C1	MC	MC/C1	*P*	C1	MC	MC/C1	*P*
45	10.6	10.0	0.95	ns	1.5	0.9	0.60	ns
45	10.8	10.0	0.93	ns	1.5	1.9	1.27	ns
56	11.3	10.1	0.89	ns	1.2	1.1	0.92	ns
72	11.0	9.6	0.87	ns	2.0	1.0	0.50	*
72	10.6	9.9	0.94	ns	2.0	0.9	0.45	*
91	11.0	9.9	0.90	ns	2.2	1.3	0.59	*
180	12.0	9.9	0.83	*	2.9	1.8	0.62	*
384	11.3	9.6	0.85	**	1.9	1.4	0.74	ns
392	11.3	9.7	0.86	**	2.0	1.1	0.55	**
493	11.1	9.2	0.83	***	2.0	0.7	0.35	***
495	11.2	9.4	0.84	***	2.0	1.2	0.60	**
510	11.1	9.7	0.88	*	2.5	1.4	0.56	**
Total	11.2	9.7	0.86	***	2.1	1.3	0.61	***

Note: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, nonsignificant.

100 crosses were evaluated simultaneously (Table 3). The reduction in inbreeding was not significant when the number of crosses dropped from 91 to 45; however, when 180–510 crosses were evaluated simultaneously, the reduction in inbreeding was highest and significant.

Discussion

This study showed that the MC scheme was the most efficient in reducing mean inbreeding of the next generation when compared with the other mating schemes applied to coho salmon at the CMG. Other authors have obtained similar results on the efficiency of the MC method for reducing inbreeding both over short (Weigel and Lin 2000) and medium (Sanchez et al. 1999) terms. Also, in agreement with various theoretical predictions (Grundy et al. 1994; Caballero et al. 1996; Sonesson and Meuwissen 2000), the random mating schemes of selected individuals were less efficient in reducing inbreeding even when avoiding mating FS and HS individuals in each generation. In spite of this evidence, previously described random mating schemes are still commonly used in selective breeding programs (Pante et al. 2001*a*; Argue et al. 2002; Bolivar and Newkirk 2002).

The MC scheme reduced the variance of inbreeding between 40% and 60% in relation to the other schemes studied, producing families that were more homogeneous among themselves with regard to inbreeding. Similar results were obtained by Sanchez et al. (1999), who found that the variance of inbreeding was reduced by one to seven times in comparison with random mating of selected individuals. This is a desirable situation in the sense that families having high levels of inbreeding within each generation will not be produced, reducing the probability of inbreeding depression being expressed (Gjerde et al. 1983; Su et al. 1996; Pante et al. 2001*b*).

In the present study, the evaluations were carried out under the conditions of two restrictions that limited the number of crosses tested simultaneously. The reproductive restriction limited the number of breeders evaluated simultaneously to those successfully spawned on a given day, and the practical restrictions in the handling of the fishes prevented a large number of them from being spawned and evaluated simultaneously. Limited by these two restrictions, only between 45 (5 males and 9 females) and 510 (17 males and 30 females) crosses could be evaluated in 2002. This was lower than the number of crosses potentially carried out at CMG on any given day (30 males and 150 females, 4500 possible crosses) and much lower than the total of theoretically possible crosses if all of the available fishes could have been spawned simultaneously (14 630 possible crosses). At least two alternative forms of spawning management could be adopted to avoid the limitations on the number of breeders simultaneously evaluated and to increase the efficiency of the minimal co-ancestry scheme. These include a reduction of the spawning period to a few days by the use of hormonal induction and the preservation of semen from early-spawning males to be used later in the fertilization process. Both techniques, especially the first one, have been incorporated in Chilean culture farms and may be efficiently utilized in the short term in genetic selection programs applied to salmonids.

Practical application of MC scheme was more time consuming than the two mating schemes previously utilized at the CMG. The MC method requires in situ (each day of spawning) construction of matrices of co-ancestry among all of the possible crosses of the breeders selected, as well as matrices necessary to adapt the selection of optimal crosses for each spawning day to a linear programming context (optimization by Hiper Lindo program, Lindo Systems Inc. 1985). In the first step, PEDIGREE VIEWER program version 4.9 (B. Kinghorn and S. Kinghorn, University of New England, Armidale, New South Wales, Australia) was used to calculate the co-ancestry of all possible crosses, but the second step was made manually. In fact, this procedure delayed the fertilization of green eggs by 2 or 3 h in comparison with R or C1 schemes. In the last step, however, the use of a computer program to adapt the selection of optimal crosses could reduce the time consumed in this period.

In this study, the comparisons among mating schemes were made after selection had been carried out and on the same level of expected genetic response; therefore, the selection and mating decisions were made independently. Under these conditions, the MC mating scheme can delay the inbreeding accumulation in the short term but does not reduce it permanently because the genetic contribution of each parent remains constant between mating schemes and the effective population size is not modified. Thus, in the long term, two populations with similar effective population size but different mating scheme should reach the same inbreeding level. Nevertheless, the MC scheme can be improved (e.g., reducing inbreeding permanently) if selection and mating decisions are simultaneously made. In the context of linear programming, an objective function should be constructed to maximize the genetic response, with a restricted inbreeding rate (Sanchez et al. 1999). However, in this case, it is expected that a compromise between the genetic response and the inbreeding rate exists where looking for a low rate of inbreeding will produce a smaller genetic response (Villanueva et al. 1996).

The MC scheme, shown to be a useful tool in delaying the accumulation of inbreeding in the short term, also reduced the variance of the inbreeding, thus limiting the early expression of inbreeding depression. These results were obtained even when the tests were carried out on very small groups of possible crosses (45–1316) because of reproductive and practical limitations. Although the MC scheme was more time consuming than the two mating schemes previously used at the CMG (R mating of selected individuals and C1 mating), we recommend this scheme to carry out crosses in each generation.

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Definitive identification of fatty acid constituents in marine mammal tissues

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Abstract: Analyses of fatty acid constituents in a sample of bowhead whale (*Balaena mysticetus*) blubber were conducted using a method that has not been applied before to studies of lipids in marine mammals. It involves creation and analysis of nitrogen (picolinyl) ester derivatives of the fatty acids followed by combined gas chromatography – mass spectrometry for verification of structures. Use of this approach allowed the structural confirmation of 45 different fatty acids in the blubber sample. The traditional method of methyl ester derivatization of fatty acids followed by gas chromatography – mass spectrometry analyses provides more component characterization than analyses by gas chromatography – flame ionization detection, but not enough to effectively differentiate double bond isomers or branched compounds. Although the novel approach is time intensive, we recommend that it be employed for studies where the precise identification and confirmation of fatty acids is important.

Résumé : L'utilisation d'une méthode qui n'a jamais encore été employée pour étudier les lipides des mammifères marins nous a permis d'analyser les composantes des acides gras dans un échantillon de lard de la baleine franche boréale (*Balaena mysticetus*). La méthode consiste en la production et l'analyse d'esters azotés (pycolinyliques) dérivés des acides gras, suivies d'une vérification des structures à l'aide d'une combinaison de chromatographie en phase gazeuse et de spectrométrie de masse (GC–MS). Cette méthode a confirmé par analyse des structures la présence de 45 acides gras différents dans l'échantillon de lard. La méthode courante de dérivatisation d'esters méthyliques d'acides gras suivie par des analyses GC–MS produit une meilleure caractérisation des composantes que les analyses par chromatographie en phase gazeuse combinée à une détection par ionisation de flamme (GC–FID), mais elle ne permet pas de différencier de façon concrète les isomères à doubles liens ou les composantes à chaînes latérales. Bien que notre nouvelle méthodologie exige beaucoup de temps, nous en recommandons l'usage dans les études qui requièrent des identifications et des confirmations précises des acides gras.

[Traduit par la Rédaction]

Introduction

Analyses of the fatty acid constituents of blubber and milk from marine mammals have recently received considerable attention as a potentially useful means of assessing feeding ecology of these animals (Bowen and Siniff 1999). In particular, it has been suggested that fatty acid signature analysis can provide great insight into food preferences and dietary shifts in marine mammals (e.g., Iverson et al. 1997*a*, 1997*b*; Lea et al. 2002). This finding has greatly interested members of the marine mammal research community, since traditional methods of assessing feeding ecology, such as gut content analyses and observations of behavior, can have significant bias (Bowen and Siniff 1999). However, some have questioned the efficacy of the techniques and interpretations of results of the fatty acid signature analyses (Grahl-Nielsen 1999).

Analyses of fatty acids in tissues can provide other insights into the functions of living systems besides assessment of feeding ecology. Pond (1996), for example, noted the possibility that changes in fatty acids in adipose depots surrounding lymph nodes modulate (via paracrine processes) the activity of the nodes. In addition, there is potential utility of documenting changes in fatty acid constituents as a clinical diagnostic marker (biomarker) of stress (Grahl-Nielsen and Barnung 1985). Regardless of the research questions being raised, it is vital that the techniques that are used permit as complete identification as possible of the fatty acid constituents present in tissues and organs.

Pond (1996) underscored the great diversity of lipids. That diversity stems in large part from the fact that the fundamental building blocks for lipids can include more than 1000 fatty acids of natural origin with a significant number of permutations for carbon chain length, numbers of double bonds, branches, and other features (Gunstone 1994; Christie 1997).

In this paper, we provide the results of fatty acid analyses of bowhead whale (*Balaena mysticetus*) blubber using picolinyl ester derivatization followed by analysis using combined gas chromatography – mass spectrometry (GC–MS). We compare these results with what could be determined using analyses of the same tissues by methyl ester derivatization and subsequent GC–MS analysis. We demonstrate that GC–MS analyses of picolinyl esters permit a more rigorous identification of the suite of fatty acids in a sample

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than do similar analyses of fatty acid methyl esters (FAMEs) by providing conclusive mass spectral structural confirmation of components. Therefore, this approach offers great promise for scientists interested in questions regarding feeding ecology, immune system modulation, stress biomarkers, and other topics in marine mammals and other species.

Methods

Assessment of analytical methods

Assessment of fatty acid components of lipids has been done routinely for several decades. The most common approach, and one that has been applied to lipid research on marine mammals, has involved the conversion of extracted lipids to methyl ester derivatives, usually via the catalyst boron trifluoride in methanol. However, in some cases, the use of this method not only can cause a significant loss of polyunsaturated fatty acids but can also create unwanted side reactions that produce confounding artifacts. These artifacts can coelute with fatty acids, producing superfluous peaks that can be misinterpreted as or camouflage a legitimate fatty acid peak (Heckers et al. 1977; Christie 1989).

After derivatization, fatty acids are commonly analyzed using gas chromatography – flame ionization detection (GC–FID) and identified by comparing retention times of sample components with those of authentic standards (Iverson et al. 1997*a*, 1997*b*). However, there are limitations to this approach. Since GC–FID identifies fatty acids by assessing only their retention times, based on retention times of standards and an expected elution pattern predicated on the polarity of the GC, this instrument can only provide tentative identifications of the fatty acids for which standards exist. Even tentative identification can be confounded by the possibility of more than one fatty acid coeluting at the same or very similar retention time. In addition, samples may have non-fatty-acid residues such as sterols and fatty alcohols that can elute with the same retention time as a fatty acid. Use of column chromatography cleanup can eliminate some of the false positive peaks and thereby increase confidence in identifications.

GC–MS can be used in lieu of GC–FID to improve the extent to which fatty acids may be distinguished from other compounds. When FAMEs are ionized, distinctive ions, such as the base ion m/z = 74 (a classical indication of the "McLafferty rearrangement ion" characteristic of FAMEs), are formed. The molecular ion (M^+) indicates the molecular mass of the fatty acid and provides information on carbon chain length and numbers of double bonds. Prominent fragments of m/z = 43, observed in saturates, and m/z = 41, observed in both monounsaturates and polyunsaturates, can also be used as further diagnostic clues for some molecular interpretation.

The analyses of FAMEs, even via GC–MS, have inherent problems, however, in determining some detailed structural information for fatty acids. During ionization, migration of double bonds along the aliphatic carbon chain occurs such that the resulting mass fragments provide little indication of the bond's original position, making a precise identification of the fatty acid unlikely (Christie 1989).

We have employed a different sample preparation technique that permits structural determination of fatty acids present in a sample. Although a few researchers have applied this technique for analyses of marine invertebrates (e.g., Rezanka and Dembitsky 1993; Ando et al. 1998), fish (e.g., Ratnayake et al. 1989; Joh et al. 1995), laboratory rodents (e.g., Grav et al. 1994), and bacteria (Carreto et al. 1996), its primary application has been in the field of agricultural research, mainly by European scientists (Brechany and Christie 1992; Christie 1997; Dobson et al. 1997). Despite the application of this technique in certain fields, its availability in the literature, and its demonstrated effectiveness, it does not appear that this technique is being applied to the determination of fatty acid composition in marine mammals.

In this method, the carboxyl group is derivatized with a reagent containing a nitrogen atom in the form of a pyridine ring. The picolinyl ester created with this technique is a stable fatty acid molecule that does not undergo double bond migration when ionized by GC–MS. Resultant fragments occur at sites of functional groups such as double bonds or branches and possess clear diagnostic features (Christie 1998). By investigating the fragments, one can reconstruct most molecules and know the exact placement of not only the double bonds but also branch chains, without the need for authentic standards.

Given the inability of GC–FID or GC–MS to unambiguously identify branched or unsaturated fatty acids derivatized via methyl esters, this derivatization technique may be less than optimal. This limitation affects the ability to answer specific questions about fatty acid constituents and changes therein and may limit the array of questions that may be effectively addressed.

Sample analyzed for this study

Analyses were conducted in triplicate on blubber taken from bowhead whale No. 98B23, a male measuring 11.7 m long that was obtained and archived by the North Slope Borough Department of Wildlife Management, Barrow, Alaska, in 1998. Tissues were collected under permit 932-1489-00 issued to Dr. Teri Rowles, National Marine Fisheries Service (1315 East-West Highway, Silver Spring, MD 13752, U.S.A.). Specifically, the sample was taken from the innermost region of blubber just caudal to the blowhole (a location that bowhead researchers consistently sample for a variety of blubber studies). Blubber was sent, frozen, to Mote Marine Laboratory (1600 Ken Thompson Parkway, Sarasota, FL 34236, U.S.A.) for analysis.

Extraction of lipids and derivatization to both methyl and picolinyl esters

Approximately 1.0 g of blubber was extracted employing a modified Folch extraction (Folch et al. 1957) using dichloromethane and methanol with butylated hydroxytoluene added as an antioxidant. For methyl ester dervatization, approximately 50 mg of lipid was transesterified using boron trifluoride in methanol solution and incubated at 100 °C for 1 h. The resulting organic layer was removed, evaporated, and redissolved in hexane for subsequent GC–MS analysis. Picolinyl ester derivatives were prepared via a modified

Destaillats and Angers (2002) method. Whole lipid was added to a homogenized mixture of 3-hydroxymethylpyridine and a solution of potassium *tert*-butoxide in tetrahydrofuran. The lipid mixture was then incubated at 45 °C for 30 min. The organic phase was washed with a 2.5% sodium bicarbonate solution, collected, dried over anhydrous sodium sulfate, and evaporated under a stream of nitrogen. The sample was redissolved in hexane. Further purification of the extracts was carried out using a Florisil$^{\text{TM}}$ column to eliminate ancillary compounds such as sterols, fatty alcohols, hydrocarbons, and residual derivatization compounds that have the potential to coelute with fatty acids. The sample was derivatized and analyzed in triplicate for each of the two different derivatization methods.

GC–MS analysis

All samples were analyzed with a Thermo Finnigan Polaris Q Ion Trap GC–MS equipped with a 30 m × 0.25 id × 0.25 µm film thickness DB5 fused silica capillary column with helium as the carrier gas. The mass spectrometer was scanned from mass 50 to 500 in 0.5 s at an ionization potential of 70 eV. For FAME analysis, the oven temperature was held at 65 °C for 2 min and then programmed from 65 to 165 °C at 20 °C·min^{-1} and held for 0.4 min, then from 165 to 215 °C at 20 °C·min^{-1} and held for 6.6 min, and then from 215 to 260 °C at 5 °C·min^{-1} and held for 10 min. The program rate for the picolinyl ester analysis began at 80 °C for 2 min, increased from 80 to 185 °C at 7 °C·min^{-1} and held for 5 min, then from 185 to 260 °C at 5 °C·min^{-1} and held for 5 min, and then from 260 to 325 °C at 5 °C·min^{-1} and held for 10 min.

Results

The GC–MS analyses of the picolinyl esters permitted the structural confirmation of 45 different fatty acids in the sample of bowhead whale blubber (Table 1). Of those fatty acids identified, 17 were saturates (including 10 branched compounds), 19 were monounsaturates, and 9 were polyunsaturates (Table 1).

Authentic standards are available commercially for 26 of the 45 fatty acids present in the sample (Table 1). Those 26 fatty acids in the blubber could be tentatively identified via GC–MS using FAMEs based on a comparison of their retention times and some spectral features with those of the standards. Standards were unavailable for the remaining 19 fatty acids present.

It is not practical to provide a detailed comparison of the results of the two different analytical techniques for each of the 45 the fatty acids identified in this study. However, the problems with structural confirmation of FAMEs, as well as the ability to clarify and confirm structure using picolinyl esters, are demonstrated by the following examples:

Figures 1–4 contain mass spectra for two common monounsaturated fatty acids, 18:1n7 and 18:1n5, derivatized as both methyl and picolinyl esters from the same whale blubber sample. Spectra from the FAMEs for 18:1*n*7 and 18:1*n*5 (Figs. 1 and 2, respectively) have some information useful for fatty acid characterization. The molecular ion *m*/*z* = 296 and the ion *m*/*z* = 264, created from loss of methanol from the molecule [M-32]$^+$, are among some diagnostic clues that indicate that these are 18:1 FAMEs (Figs. 1 and 2). However, the spectra from these two 18-carbon monounsaturated FAMEs, or from any of the other possible 18:1 FAME isomers, are virtually indistinguishable from each other. Other than confirmation that they are indeed FAMEs, with a particular chain length and number of double bonds (as determined by the molecular ions), little other information can be obtained from these spectra to allow a more definitive identification.

Table 1. Fatty acids identified from blubber samples of a bowhead whale using gas chromatography – mass spectrometry analysis of picolinyl esters.

Fatty acids with available standards	Fatty acids without available standards
12:0	14:1*n*7
13:0	4,8,12-trimethyl-16:0
14:1*n*5	15:1*n*7
14:0	15:1*n*6
iso 15:0	16:4*n*1
anteiso 15:0	16:1*n*11
15:0	16:3*n*4
16:1*n*9	16:1*n*5
16:1*n*7	2,6,10,14-tetramethyl-19:0
16:2*n*4	Anteiso 17:0
16:0	17:1*n*8
iso 17:0	3,7,11,15-tetramethyl-20:0
17:0	Anteiso 18:0
18:4*n*3	18:1*n*5
18:1*n*11	9-me-20:0
18:1*n*9	20:1*n*11
18:1*n*7	21:5*n*3
18:0	10-me-22:0
20:4*n*6	22:1*n*7
20:5*n*3	
20:1*n*9	
20:1*n*7	
22:6*n*3	
22:5*n*3	
22:1*n*11	
22:1*n*9	

Note: Authenticated standards exist for 26 of the 45 (58%) fatty acids identified. We attempted an exhaustive search for fatty acid methyl ester standards that are available. It is possible that particular laboratories or supply companies of which we are unaware may possess a small number of additional authentic fatty acid standards. Fatty acids are designated by shorthand convention nomenclature of carbon chain length:number of double bonds and location (*n*–*x*) of the double bond closest to the terminal methyl group.

In comparison, the spectra from the same two fatty acids derivatized to picolinyl esters have a number of diagnostic ions that allow for the reconstruction of the fatty acid molecules (Figs. 3 and 4). In both figures, the spectra have mass fragments corresponding to the systematic cleavage of "pieces" of the picolinyl ester that can be reassembled to reproduce the fatty acid in question. Prominent masses of *m*/*z* = 92, 108, and 151 contain the pyridine ring and other

Fig. 1. Spectra resulting from the mass spectral analysis of the methyl ester for fatty acid 18:1*n*7. The two diagnostic ions indicated permit the identification of a fatty acid molecule with 18 carbons and one double bond.

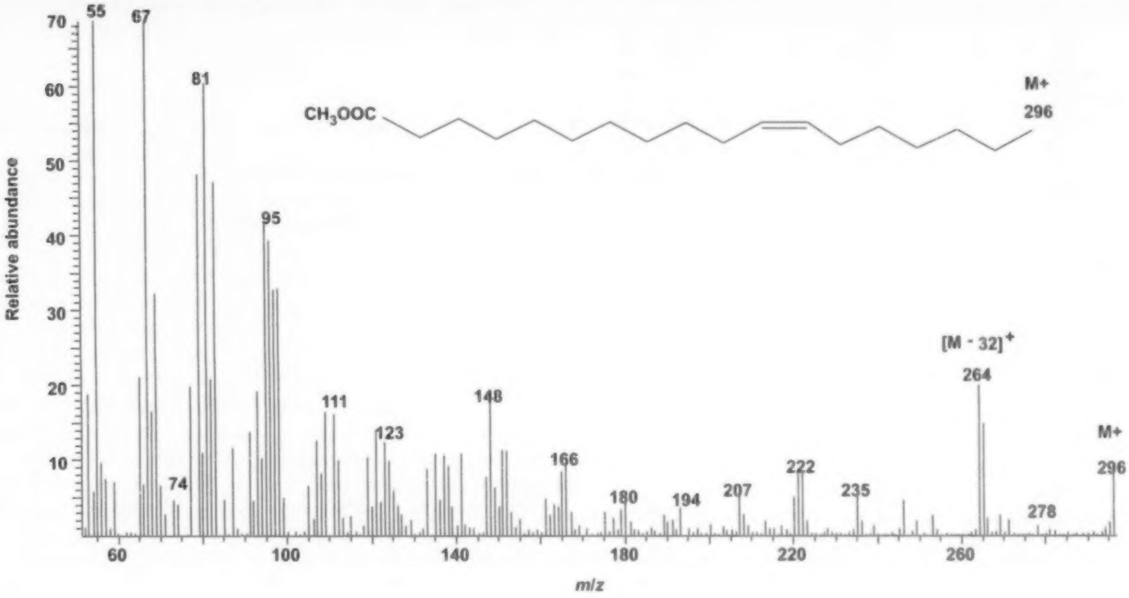
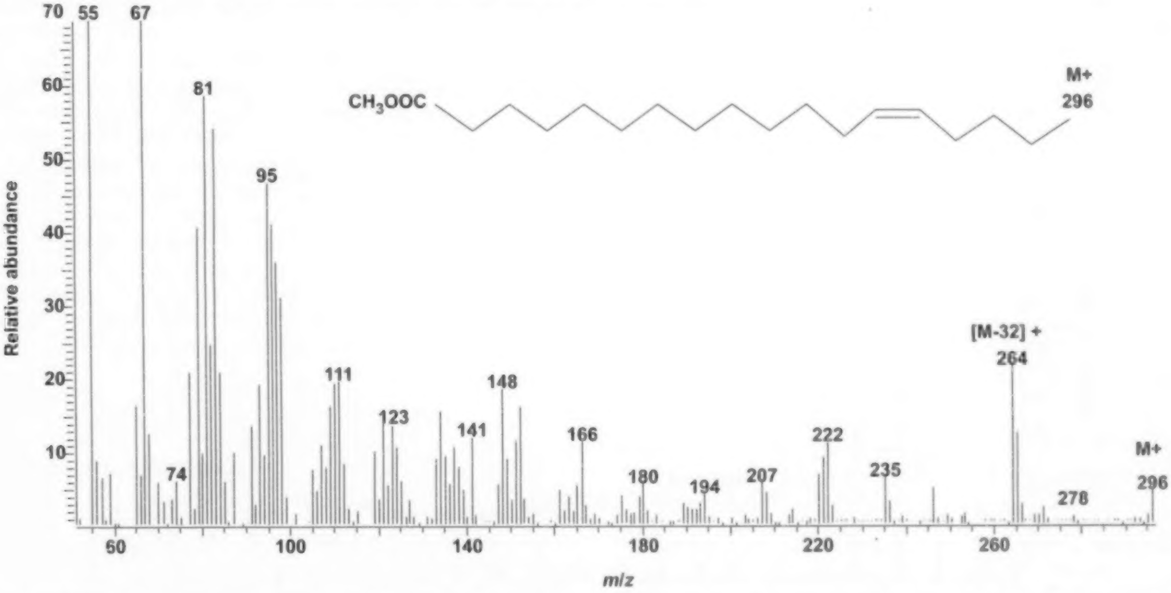


Fig. 2. Spectra resulting from mass spectral analysis of the methyl ester for fatty acid 18:1*n*5. The two diagnostic ions indicated permit the identification of a fatty acid molecule with 18 carbons and one double bond.



components of the carboxyl group, indicative of picolinyl esters; these are used to distinguish fatty acids from any non-fatty-acid peak. The fragmentation pattern of the other functional groups created during ionization of these molecules is even more useful. In these particular examples of 18:1*n*7 (Fig. 3) and 18:1*n*5 (Fig. 4), the molecular ion of m/z = 373 is present in each spectrum. This mass, corresponding to the molecular mass of the compound, specifies (as in the methyl esters) the carbon chain length and number of double bonds. Beyond that, the spectra also denote the location of the double bond(s) in the fatty acid analyzed. There is a loss of 15 atomic mass units for the cleavage of the terminal methyl group and a loss of 14 atomic mass units for each subsequent methylene group cleaved from the carbon chain. When a double bond is reached during this fragmentation process, there is an increase in the intensity of the

Fig. 3. Spectra resulting from mass spectral analysis of the picolinyl ester for fatty acid 18:1*n*7. The molecular ion of m/z = 373 provides information on chain length (18 carbons) and number of double bonds (one). Additional diagnostic ions allow interpretation of the position of the double bond.

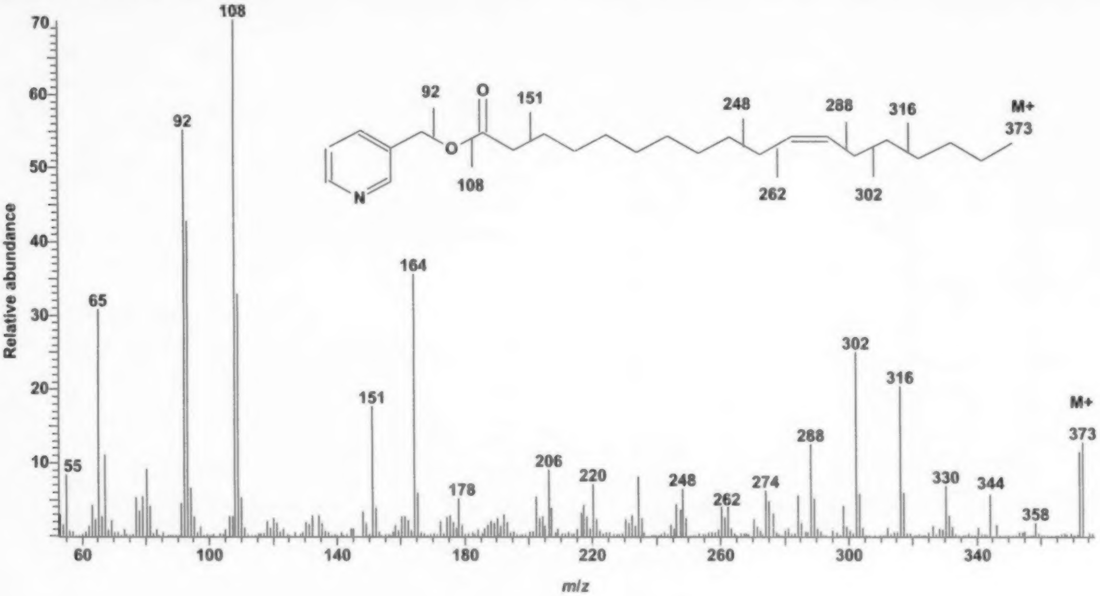
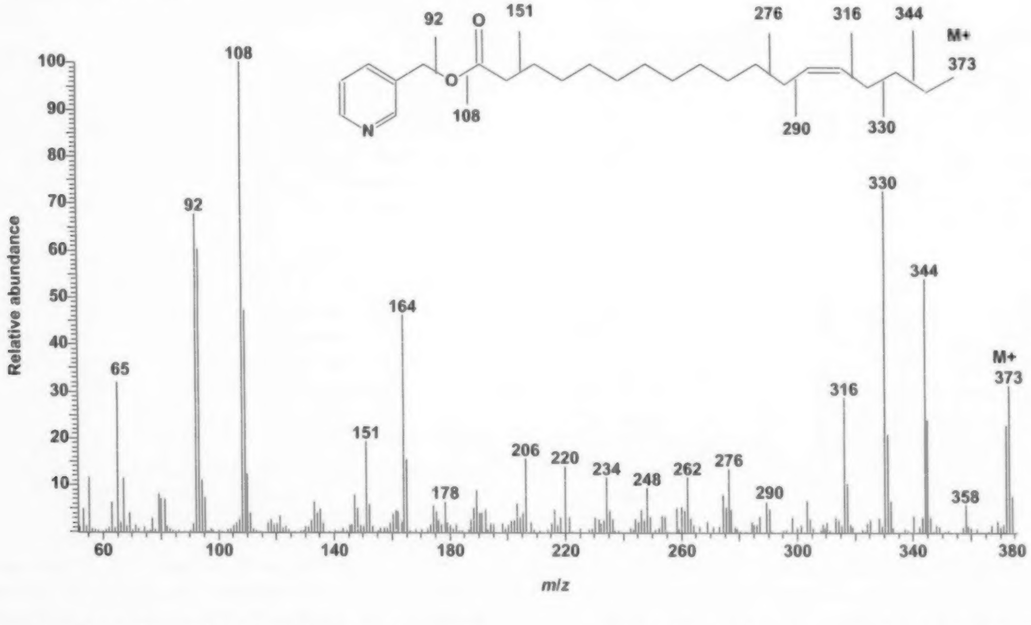


Fig. 4. Spectra resulting from mass spectral analysis of the picolinyl ester for fatty acid 18:1*n*5. The molecular ion of m/z = 373 provides information on chain length (18 carbons) and number of double bonds (one). Additional diagnostic ions allow interpretation of the position of the double bond.



two ions on the distal side of the double bond, each a methylene group apart, at m/z = 302 and 316 for 18:1*n*7 or m/z = 330 and 344 for 18:1*n*5 (Figs. 3 and 4, respectively). This pair of indicator ions is followed by a loss of one more methylene group (14 atomic mass units) and then a subsequent gap of 40 atomic mass units for the double bond and associated methylene group, thereby indicating quite elegantly the location of the double bond(s).

The same stepwise assembly process, using diagnostic ions created during ionization, can be applied to picolinyl esters of complex, branched fatty acids and polyunsaturated fatty acids. Thus, utilizing the picolinyl ester technique allows a more complete characterization of fatty acid structures for identification.

Discussion

Fatty acid constituents of tissues can be determined easily and with a high degree of confidence through GC–MS analysis of the picolinyl ester derivatives of the fatty acids. In fact, we demonstrate that use of this technique to assess bowhead blubber permits the confirmation of 45 fatty acids, whereas use of the most generally applied technique would only allow the tentative identification of 26 (58%) of those fatty acids in the sample. The relative levels of resolution that can be achieved using different protocols and instruments were discussed in the Methods section.

The preferred technique was developed over two decades ago and has been validated well, especially by lipid chemists engaged in crop research in Europe. Although it has been well described in the literature (Harvey 1982; Christie 1998; Destaillats and Angers 2002), the method has not, to the best of our knowledge, been applied to studies of marine mammals.

This method may be viewed as having certain disadvantages. For example, it is more costly, in terms of time, than other approaches that have been commonly used. Especially as researchers begin using the new technique, the "reassembly" of the "pieces" of a particular fatty acid that are created by ionization during mass spectrometry requires considerable time and a painstaking approach. In addition, laboratories interested in applying the technique may lack a GC–MS, a rather costly instrument.

Nonetheless, the advantages of performing GC–MS analyses of picolinyl esters far outweigh the disadvantages. Scientists engaged in fatty acid analyses have commonly reinforced the paramount importance of being able to identify as many components as possible, with as high accuracy as possible, to address questions relating to feeding ecology or other subjects. That being the case, we feel that the analysis of picolinyl esters via GC–MS should be done whenever possible.

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Twentieth century water quality trends in Minnesota lakes compared with presettlement variability

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Abstract: A diatom-based transfer function was used to reconstruct water chemistry before European settlement in 55 Minnesota lakes. The lakes span three natural ecoregions, which differ in their history of land use, as well as in surficial geology, climate, and vegetation. Postsettlement trends were compared with water chemistry change reconstructed from two presettlement core sections (circa 1750 and 1800) as a measure of natural variability. Presettlement water quality changes were generally small and nondirectional in all three ecoregions. In contrast, half of the urban lakes showed a statistically significant increase in chloride, whereas 30% of urban and 30% of agricultural region lakes record a statistically significant increase in total phosphorus between 1800 and the present. These changes, which are attributed to road salt and nutrient runoff, are strongly correlated with the percentage of watershed area that is developed (residential or urban) in the case of chloride increases and the percentage of developed (metropolitan areas) or agricultural (agricultural areas) land in the case of nutrient increases. Water quality has changed little since 1800 for lakes in the forested regions of northeastern Minnesota. The few changes that are seen in this region are likely related to natural variations in climate or catchment soils.

Résumé : L'utilisation d'une fonction de transfert basée sur les diatomées nous a permis de reconstituer la chimie des eaux dans cinquante-cinq lacs du Minnesota avant l'établissement des européens. Les lacs se répartissent dans trois écorégions naturelles qui diffèrent par l'utilisation que l'on a fait des terres dans le passé, ainsi que par la géologie de surface, le climat et la végétation. Nous avons comparé les tendances qui sont apparues après la colonisation avec les changements estimés à partir de deux sections de carottes datant d'avant la colonisation (vers 1750 et 1800) qui représentent la variabilité naturelle. Avant la colonisation, les changements de la qualité de l'eau dans les trois écorégions étaient généralement faibles et sans tendance marquée particulière. En revanche, entre 1800 et l'époque actuelle, la moitié des lacs urbains affichent une augmentation statistiquement significative des chlorures et 30 % des lacs urbains et 30 % des lacs des régions agricoles subissent une augmentation statistiquement significative du phosphore total. Il existe une forte corrélation entre ces changements, qui sont attribuables à l'apport du sel routier et de nutriments par ruissellement, avec le pourcentage des terres du bassin versant qui sont urbanisés (résidentielles ou urbaines) dans le cas de l'augmentation des chlorures ou alors développées (régions métropolitaines) ou exploitées pour l'agriculture (zones agricoles) dans le cas de l'augmentation des nutriments. Dans les régions forestières du nord-est du Minnesota, la qualité des eaux des lacs a peu varié depuis 1800. Les quelques changements observés dans cette région sont vraisemblablement reliés aux variations naturelles du climat ou des sols du bassin versant.

[Traduit par la Rédaction]

Introduction

Human activities can have profound effects on lake ecosystems. These impacts have been widely documented in a number of limnological (Edmondson and Lehman 1981; Lewis et al. 1984; Jassby et al. 1995) and paleolimnological (Engstrom et al. 1985; Fritz et al. 1993; Hall and Smol 1996) studies of individual lakes or small clusters of lakes, as well as in a limited number of larger-scale studies that address regional trends in water quality. These large regional studies were undertaken to evaluate lake acidification (Kingston et al. 1990; Cumming et al. 1992), forestry practices in western Canada (Laird and Cumming 2001), or limnological change in the eastern United States attributable to multiple environmental stressors, including diverse human activities (Rohm et al. 1995; Dixit et al. 1999; Siver et al.

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1999). Little synoptic work has been undertaken in central North America, where widespread agricultural and forestry practices, as well as mining, may have had significant impacts on aquatic ecosystems.

Minnesota is geographically diverse, and different parts of the state vary in their history of settlement and land use. Pollution is a problem in many of Minnesota's aquatic ecosystems as a result of runoff from agriculture and urban development in the southern part of the state, as well as atmospheric transport of pollutants throughout the region (Swain et al. 1992). Paleolimnological studies have been used previously to reconstruct human impact on regional lakes (Bradbury 1975; Bradbury and Winter 1976; Brugam and Speziale 1983); however, these earlier studies involved qualitative, not quantitative (Charles and Smol 1994), assessments of trophic change and included only a small selection of lakes. Moreover, strong environmental gradients characterize the state, and short-term shifts in climate have the potential to affect lake systems and hence water quality (e.g., Schindler et al. 1996; Bradbury et al. 2002). Hence, it is important to evaluate human-induced limnological change against a background of natural environmental variability.

Only a very small number of regional paleolimnological studies have analyzed multiple lakes and multiple time intervals in evaluating human impacts on lacustrine systems (Cumming et al. 1994; Laird and Cumming 2001). The majority have compared a single sample from presettlement times with modern conditions and assumed that the observed change is largely a result of human modification of the environment. However, it is difficult to disentangle the impact of natural environmental variability from impacts attributable directly to humans based on a simple comparison of two points in time. Here, we evaluate the magnitude of human impacts relative to natural forcing in a suite of 55 lakes from three different ecoregions of Minnesota variously affected by human activities. We compare the magnitude of twentieth century change with change over a 50-year period in the eighteenth century, before European settlement of the region. This comparison is based on a diatom transfer function developed from regional lakes (Ramstack et al. 2003), which is used to generate quantitative reconstructions of water chemistry conditions from sediment cores.

Materials and methods

Study area

The 55 lakes in this study occur in three of Minnesota's ecoregions (Omernik 1987): Northern Lakes and Forests (NLF), North Central Hardwood Forests (NCHF), and Western Corn Belt Plains (WCBP), as well as the Twin Cities metropolitan (Metro) region (Appendix A, Table A1; Fig. 1). These ecoregions span gradients in vegetation, precipitation, geology, and land use that run from northeast to southwest across the state. The lakes were chosen for a study on mercury deposition across Minnesota (Engstrom et al. 1999), with the addition of five lakes from agricultural regions in southern Minnesota (those in the WCBP ecoregion). The lakes were not chosen randomly for the previous study; however, they are qualitatively representative of the larger population of lakes in each of the ecoregions.

The study lakes range in size from 5.3 to 644.8 ha, although only two of the lakes (Diamond and Wilson) exceed 200 ha (Appendix A, Table A2). The range of lake surface areas is similar for the Metro and NLF groups (median area = 38 and 47 ha, respectively) and only slightly larger for the NCHF and WCBP groups (median = 76 ha). Maximum lake depth for the entire data set ranges from 2.4 to 42.7 m, with WCBP lakes being somewhat shallower than those in the other three groups (median = 13.1, 10.5, 11.4, and 8.2 m for Metro, NLF, NCHF, and WCBP, respectively).

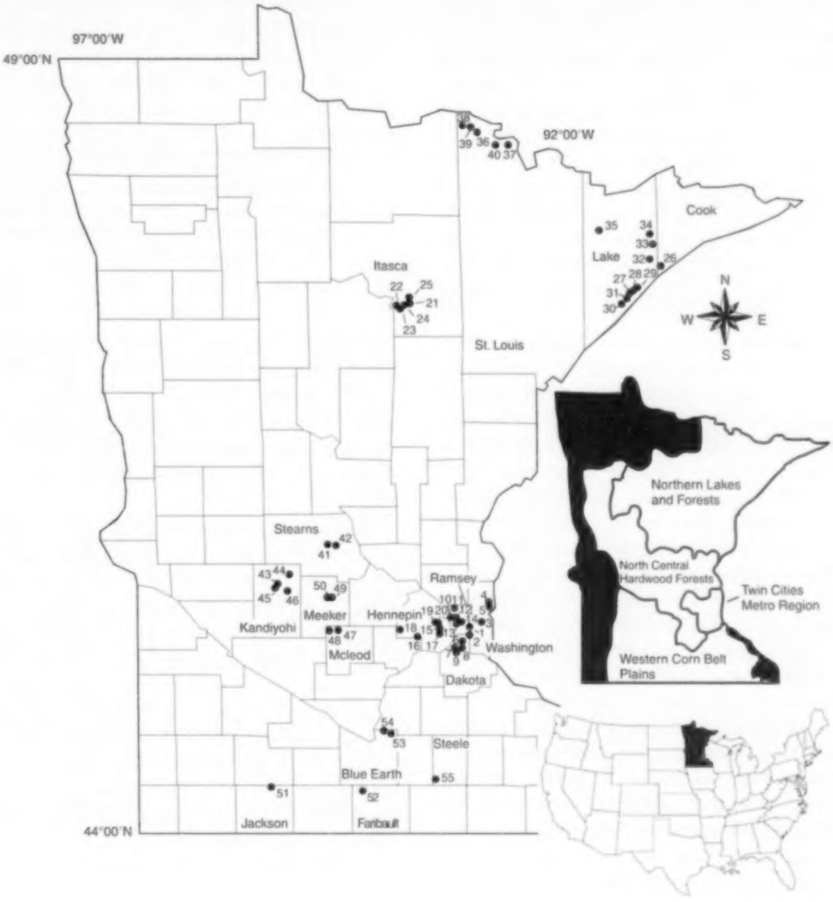
Total catchment area (excluding lake surface) for the 55 study sites ranges from 32 to 4028 ha (Appendix A, Table A2), whereas the ratio of total catchment to lake surface ranges from 1.12 to 120.8 with a median value of 5.57 and an interquartile range (IQR) of 10.74. About half of the 55 lakes receive drainage from upstream lakes within their catchments. The primary catchment, which excludes that portion of the watershed above the outflow from these lakes, ranges from 18 to 1850 ha and in most cases is less than half that of the total catchment. Additional information on the lakes can be found in Ramstack et al. (2003) and Engstrom et al. (1999).

The NLF region is dominated by mixed conifer–hardwood forest, with only small tracts of land permanently cleared for agriculture and iron mining. Urban development in this portion of the state is limited; however, some lakeshores are lined with resorts and cabins. Most lakes are small and deep and are oligotrophic to mesotrophic (Appendix A, Table A1). Soils are generally thin and overlie base-poor crystalline bedrock of the Canadian Shield. Twenty of the study lakes are in this ecoregion: five are clustered in carbonate-rich terrain near the city of Grand Rapids (lakes 21–25), five are located within Voyageurs National Park near the Canadian border (lakes 36–40), six are located within the highlands along the north shore of Lake Superior (lakes 26–31), and the remaining four are scattered widely across the Superior National Forest (lakes 32–35) (Fig. 1).

The NCHF ecoregion is characterized by a mosaic of land uses (Heiskary et al. 1987). Agricultural and forested use predominates in the west-central portion of the ecoregion, whereas the Metro area in the east is dominated by urban, wetland, and forested areas (Engstrom et al. 1999). The lakes in this region range from oligotrophic to hypereutrophic (Appendix A, Table A1). In contrast with the NLF region, most of these lakes lie in calcareous glacial drift and consequently have high alkalinities. Forty-four percent of the lakes in this ecoregion are considered unsuitable for contact recreation because of low water quality (Minnesota Pollution Control Agency 2000). Annual evaporation generally exceeds precipitation, and lake levels often fluctuate seasonally and from year to year. Ten of the study lakes are located within this region, and half of these have large, agriculturally dominated watersheds and low water quality. The other five lakes are situated in smaller and more forested catchments and have higher water quality. All but two of the lakes are ringed by summer cottages and year-round homes.

The Metro region is considered part of the NCHF ecoregion but is treated separately here, as many of the lakes are affected by urban land use. Twenty of the study lakes are in this metropolitan area, and approximately half are in urban or residential neighborhoods (within 10 km of the center of

Fig. 1. Location of the 55 study lakes along with the ecoregions of Minnesota, U.S.A. (modified from Tester 1995). See Appendix A for key to lake numbers.



either Minneapolis or St. Paul). The other half is in more rural settings with a mixture of current land use, including woodlands, farms, and suburban development.

Agriculture is the primary land use in the WCBP ecoregion, and more than 80% of the land is under cultivation. The five study lakes in this region are large and shallow. As a result of rich soils, shallow lake basins, and runoff from farm fields, most lakes in this region are classified as eutrophic or hypereutrophic (Appendix A, Table A1).

Geographic information system (GIS) database

The GIS database for this study contains seven thematic layers in Arc/Info format for each of the 55 watersheds. Each layer is cast in the Universal Transverse Mercator Zone 15 projection with units in metres, the North American Datum 1983 for horizontal reference, and the Global Reference System 1980 as the selected spheroid. Watershed boundaries were initially delineated on U.S. Geological Survey 7.5-minute series topographic quadrangles for each of the study lakes digitized in ArcEdit to form the Basemap cover. The source data for the Transportation cover is the State of Minnesota BaseMap developed by the Minnesota Department of Transportation. The land cover layer contains polygons representing 10 land-use types (grassland, agland, brushland, deciduous forest, conifer forest, mixed forest, open water, wetlands, built up, and clearcut), based on a modified U.S. Geological Survey classification scheme (Anderson et al. 1976). These features were interpreted from early-spring 1991–1992 color infrared National Aerial Photography Program photographs. Stereoscopic interpretations were accomplished using an Old Delft scanning stereoscope with a 4.5 magnification setting resulting in an image scale of about 1:9000. Landscape feature classes were digitized using ArcEdit and a U.S. Geological Survey panchromatic digital orthophoto quarter quad as a background image. Approximately 50% of the watersheds in the study were spot field checked for classification accuracy.

Sediment cores, water chemistry analysis, and sample preparation

Sediment cores were obtained in the mid-1990s in conjunction with a study of mercury deposition in Minnesota (Engstrom et al. 1999). The cores were taken with a piston corer from a deep flat region of each of the lake basins. The cores were sectioned in the field and subsequently freeze dried. A chronology was established with ^{210}Pb dating using the constant rate of supply model (Appleby and Oldfield 1978). Based on the ^{210}Pb chronology and extrapolated sedimentation rates (Engstrom et al. 1999), samples of 1–5 cm in thickness and representing 2–4 years of accumulation were taken from the core top and from the intervals representing approximately 1750, 1800, and 1970.

In the modern samples, at least 400 diatom valves were counted along transects. Three hundred valves were counted in the core sections, or 10 transects, whichever occurred earlier. Krammer and Lange-Bertalot (1986–1991) and Patrick and Reimer (1966) were used to identify diatoms to species. Reconstructions were not performed on seven core samples because of low diatom concentration.

A modern diatom calibration data set developed for Minnesota lakes (Ramstack et al. 2003) was used to reconstruct water chemistry characteristics from sedimentary diatom assemblages. Weighted averaging calibration, with inverse deshrinking (Juggins and ter Braak 1992), was used to generate a transfer function to infer chloride (Cl), color, total phosphorus (TP), and pH from sedimentary assemblages. Each of these variables had a statistically significant influence ($p < 0.005$) on modern diatom distribution in the calibration data set. The strength of the transfer function is reported in terms of the squared correlation (r^2) and the root mean square error (RMSE) (Table 1). Because the same data are used to generate the model and test it, the RMSE usually underestimates the RMSE of prediction (RMSEP), and the validation step of jackknifing is used. For each lake, the transfer function was applied to a surface sediment sample representing modern (1995) conditions, a subrecent interval (approximately 1970), and the 1800 and 1750 core sections. See Ramstack et al. (2003) for additional details on the transfer function development and statistical analyses.

Results

Core diatom assemblages

Presettlement sediments from sites along the North Shore of Lake Superior (NLF ecoregion) are dominated by *Aulacoseira ambigua* (Grunow) Simonsen and *Cyclotella pseudostelligera* Hustedt (Fig. 2), species that are characteristic of oligotrophic to mesotrophic lakes (Hall and Smol 1992; Reavie et al. 1995*b*). In some recent samples (1995 and 1970) from this region, *Fragilaria construens* var. *venter* (Ehrenberg) Grunow and *Asterionella formosa* Hassall show small increases relative to *Aulacoseira ambigua*. Lakes in Voyageurs National Park, as well as the Superior National Forest, are dominated in both recent and presettlement samples by *Asterionella formosa*, *Cyclotella stelligera* Cleve and Grunow, and *Tabellaria flocculosa* (Roth) Kützing; these diatoms have TP optima that are characteristic of mesotrophic systems (Hall and Smol 1992; Reavie et al. 1995*a*, 1995*b*). However, *Cyclotella stelligera* is much less common in the down-core sections than late twentieth century sections. *Fragilaria crotonensis* Kitton, which has been associated with nutrient enrichment in lakes of low to moderate alkalinity (Bradbury 1975; Brugam 1979), is dominant in presettlement core sections from two of the five lakes clustered near Grand Rapids, as compared with four lakes in the modern samples. *Stephanodiscus minutulus* (Kützing) Cleve and Möller makes up 21% of the diatom assemblage in the 1800 core section from one of these five lakes (Loon) and 10% of the 1750 core section of another (Long). These are the only two samples from the NLF ecoregion in which *Stephanodiscus* is a significant portion of the diatom assemblage.

Table 1. Results of the weighted averaging regression.

Environmental variable	r^2	RMSE (apparent)	RMSE of prediction
log Cl	0.77	0.43	0.55
log color	0.68	0.20	0.28
log TP	0.68	0.19	0.25
pH	0.80	0.28	0.35

Note: The strength of the transfer function is reported in terms of the squared correlation (r^2), the root mean square error (RMSE) (observed – inferred), and the jackknifing root mean square error (RMSE of prediction) (modified from Ramstack et al. 2003).

In the 11 centrally located lakes of the Metro region (all now highly urbanized), small *Stephanodiscus* species, which are known to have high P requirements (Bradbury 1975; Fritz et al. 1993; Bennion et al. 1996), are much less prevalent in presettlement sediments than in the recent samples (Fig. 2). In the presettlement core sections, the species assemblages are dominated by *Aulacoseira ambigua*, *Cyclotella bodanica* var. *lemanica* (O. Müller) Bachmann, and *Cyclotella michiganiana* Skvortzow. In the nine lakes more distant from the urban core, presettlement species assemblages are dominated by *Aulacoseira ambigua*, *Fragilaria brevistriata* Grunow, and *Fragilaria construens* var. *venter*, whereas *Asterionella formosa*, *Fragilaria crotonensis*, and *Cyclotella bodanica* var. *lemanica* are more abundant in recent samples.

In what is now the agricultural part of Minnesota, some of the lakes contain presettlement diatom assemblages similar to those of the present day, whereas others are substantially different (Fig. 2). The 1800 and 1750 core sections of lakes in the WCBP ecoregion are dominated by *Aulacoseira ambigua*, *Aulacoseira granulata* (Ehrenberg) Simonsen, and small *Stephanodiscus* species. The present-day assemblages are similar; however, in a few of the lakes, there are larger percentages of mesotrophic and eutrophic planktonic taxa, including *Asterionella formosa*, *Fragilaria crotonensis*, and small *Stephanodiscus* species in the recent samples, with small declines in benthic *Fragilaria* species. Lakes in the currently agricultural areas of the NCHF ecoregion are dominated in presettlement times by small *Stephanodiscus* species, *Fragilaria construens* var. *venter*, and *Fragilaria pinnata* Ehrenberg. The recent samples are dominated by small *Stephanodiscus* species, *Aulacoseira granulata*, and *Aulacoseira ambigua*. In contrast, in currently forested parts of this ecoregion, *Fragilaria construens* var. *venter* is present in 1800 and 1750 core sections from the majority of the lakes. Other common diatoms in presettlement levels include *Cyclotella bodanica* var. *lemanica*, *Aulacoseira ambigua*, and *Aulacoseira granulata*. In the modern samples, *Fragilaria crotonensis* and small *Stephanodiscus* species dominate.

Water chemistry reconstructions

Reconstructions were made for Cl, pH, color, and TP. The results for the pre- versus post-settlement diatom reconstructions are expressed as the change from 1800 to the present (1995) (Fig. 3), and change before European settlement is expressed as the change from 1750 to 1800 (Fig. 4). For Cl, color, and TP, the reconstructions are based on log-transformed modern water chemistry data. Thus, the changes shown are log minus log values, which are expressions of the ratio of change. The RMSEP is used to evaluate the magnitude of water chemistry change. In our discussion of reconstructed water chemistry trends below, we use the term "significant" not in the strict statistical sense but for changes between two periods of time that exceed the RMSEP. Regional water chemistry means are compared for the 1750, 1800, 1970, and modern core sections (Fig. 5) (NCHF and WCBP lakes are grouped together because of the similar surficial geology and strong agricultural influence in both regions; variables that were log transformed (Cl, color, and TP) are expressed as back-transformed values).

All of the Metro region lakes show increases in Cl concentrations from 1800 to the present (10 of which exceed the RMSEP). The mean concentration has increased from 6 to 47 ppm, and the range of values has increased considerably (Fig. 5). In the NCHF and WCBP ecoregions, four lakes also show an increase in Cl that exceeds the RMSEP, whereas only one lake records a decrease. Overall, the median value for the region has risen. Three lakes in the NLF ecoregion record a significant decrease in Cl.

Lakes in the NLF ecoregion show either a decrease in pH (3 sites) or no significant change since 1800 (Fig. 3). In the other regions, the majority of lakes record an increase in pH, and only two lakes show a decrease that exceeds the RMSEP (Fig. 3). Only eight of the study lakes show a change in color since 1800. Regional trends in this variable are not apparent (Fig. 3), although no lakes in the NLF ecoregion show a significant decrease in color.

TP shows strong regional patterns (Figs. 3 and 5). Of the 20 Metro region lakes, 12 show increases in diatom-inferred TP between 1800 and today (six exceed the RMSEP); none of the lakes in this region show a significant decrease (Fig. 3). The average TP concentration for this group of lakes has increased from 23 to 35 ppb since settlement, whereas the range of values has doubled (Fig. 4). Half of the 15 lakes in agricultural areas of west-central and southern Minnesota show increases in TP from 1800 to the present (three of which exceed the RMSEP); the rest have not shown significant change (Fig. 3). In contrast, the northeastern lakes show no systematic TP changes from 1800 to the present; about half the lakes show modest declines in TP, whereas the others show modest increases or no change (Fig. 3). It is also clear from the diatom reconstructions that TP levels in southern and western Minnesota, although currently enhanced by human impacts, have always been higher than those in the northeastern part of the state (Fig. 5).

Land use and water quality change

GIS characterization of catchment land use for the 55 study lakes follows closely the general pattern for these ecoregions as a whole. The primary catchments of the Metro lakes are dominated by built-up cover types (residential and commercial), those in the NLF by forest (conifer and deciduous), and catchments in the NCHF and WCBP by agriculture (pasture and tillage) (Appendix A, Table A2). The median for built up in Metro catchments is 78%, with only two lakes (Schultz and Little Long) having less than 20% of this cover type. By contrast, 14 of the NLF lakes have catchments that are entirely undeveloped (forest or wetland), and five more are more than 80% undeveloped. Among the NCHF and WCBP lakes, 10 catchments exceed 50% agricultural land use and four others have more than 20% of this cover (Appendix A, Table A2). In the Metro area, only three lakes (Elmo, Little Carnelian, and Square, all in Washington County) have greater than 20% of their catchments in agriculture, although most lands in the Metro region were intensively farmed at one time.

Diatom-inferred water quality changes between 1800 and the present (numerically represented as the difference between present-day and 1800 values, log transformed except pH) are correlated with catchment land-use patterns within ecoregions (Table 2). Reported correlations are based on primary catchment land use (which excludes areas above the outflow from upstream lakes), although results for total catchment land use are quite similar. Among Metro region lakes, increases in TP show significant positive relationships with the percentage of catchment area devoted to built-up cover types ($r = 0.54$, $p = 0.01$) and road surfaces ($r = 0.49$, $p = 0.03$). Chloride increases are even more strongly correlated with these cover types (built up, $r = 0.71$, $p < 0.01$; roads, $r = 0.63$, $p < 0.01$). Because built-up areas have more road surfaces, these cover types are highly correlated, and their individual influences on TP and Cl increases cannot be disentangled. Nonetheless, these relationships strongly imply that the magnitude of postsettlement water quality change (TP and Cl) in the Metro region is directly related to the degree of urbanization within individual lake catchments.

Among lakes in the NCHF ecoregion, TP increases are positively correlated with the percentage of catchment devoted to agriculture ($r = 0.72$, $p = 0.02$) and conversely with the percentage of catchment remaining in forest cover ($r = -0.62$, $p = 0.06$) (Table 2). Postsettlement increases in apparent color (dissolved organic carbon (DOC)) are also related to agricultural land use ($p = 0.68$, $p = 0.03$). These results provide strong evidence that conversion to agricultural land uses has caused proportional increases in nutrient runoff and historical eutrophication of NCHF lakes. The increases in lake color, although unexpected, may be related to increased export of soil organic matter (possibly enhanced by field application of livestock manure) from catchment areas devoted to tillage agriculture.

In the small group of lakes ($n = 5$) from the WCBP ecoregion, TP increases are most strongly correlated (although not significantly because of the small sample size) with the percentage of catchment in built-up land uses, primarily lakeshore cottages and homes ($r = 0.63$, $p = 0.3$). The weak correlation with agricultural land use (as compared with NCHF lakes) results from the fact that all WCBP catchments are dominated by agriculture (60–90%).

Among the NLF lakes of northeastern Minnesota, diatom reconstructions reveal little if any changes in postsettlement water quality and only weak correlations with present-day catchment land use (Table 2). These catchments remain al-

Fig. 2. Diatom relative abundance of the most common taxa in the four analyzed core samples, arranged according to ecoregion. Presettlement samples are indicated with open bars and the postsettlement pair of samples (1970, 1995) are indicated with solid bars. For each lake, the four dates are arranged in order from oldest at the base to most recent at the top.

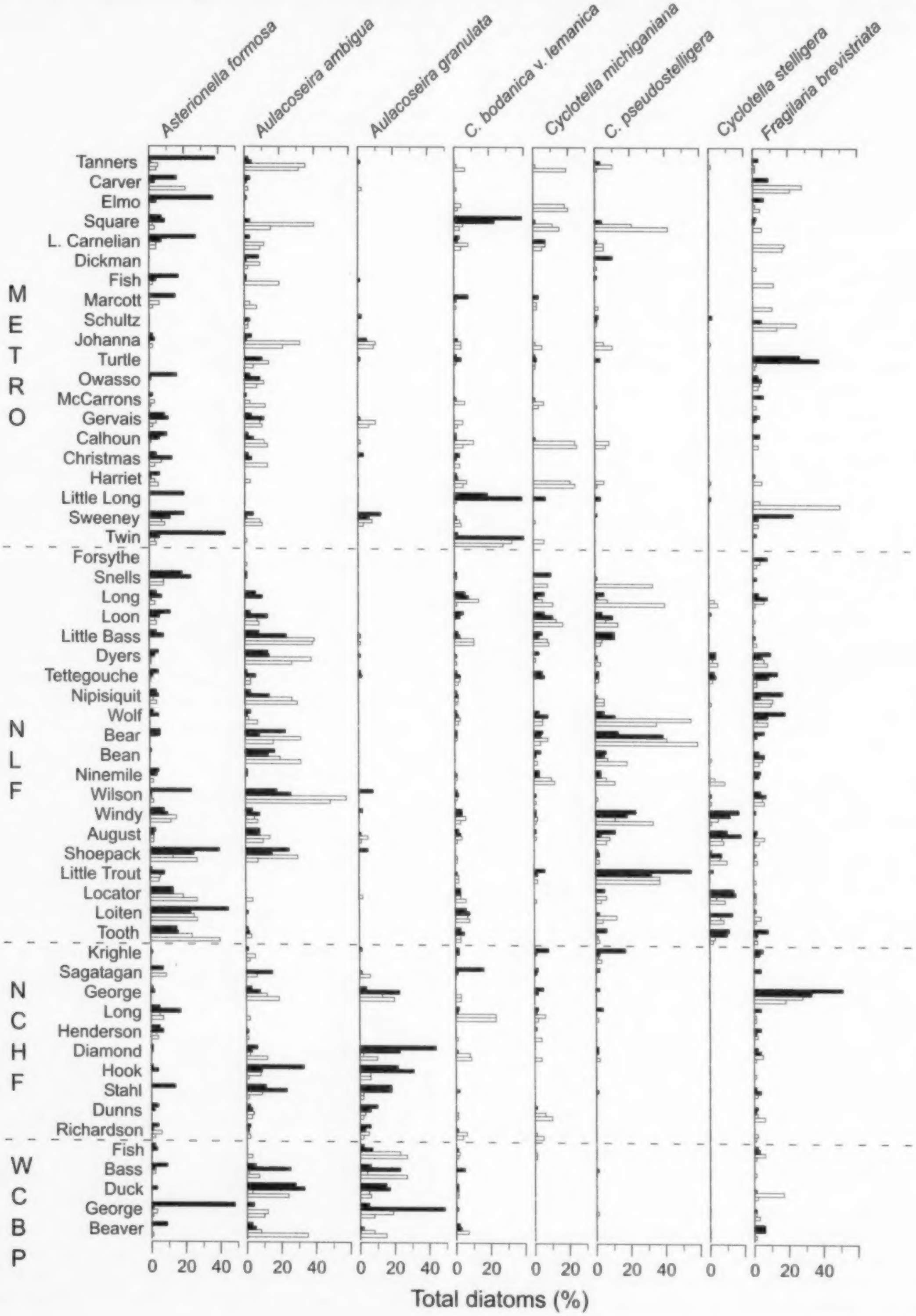


Fig. 2 (*concluded*).

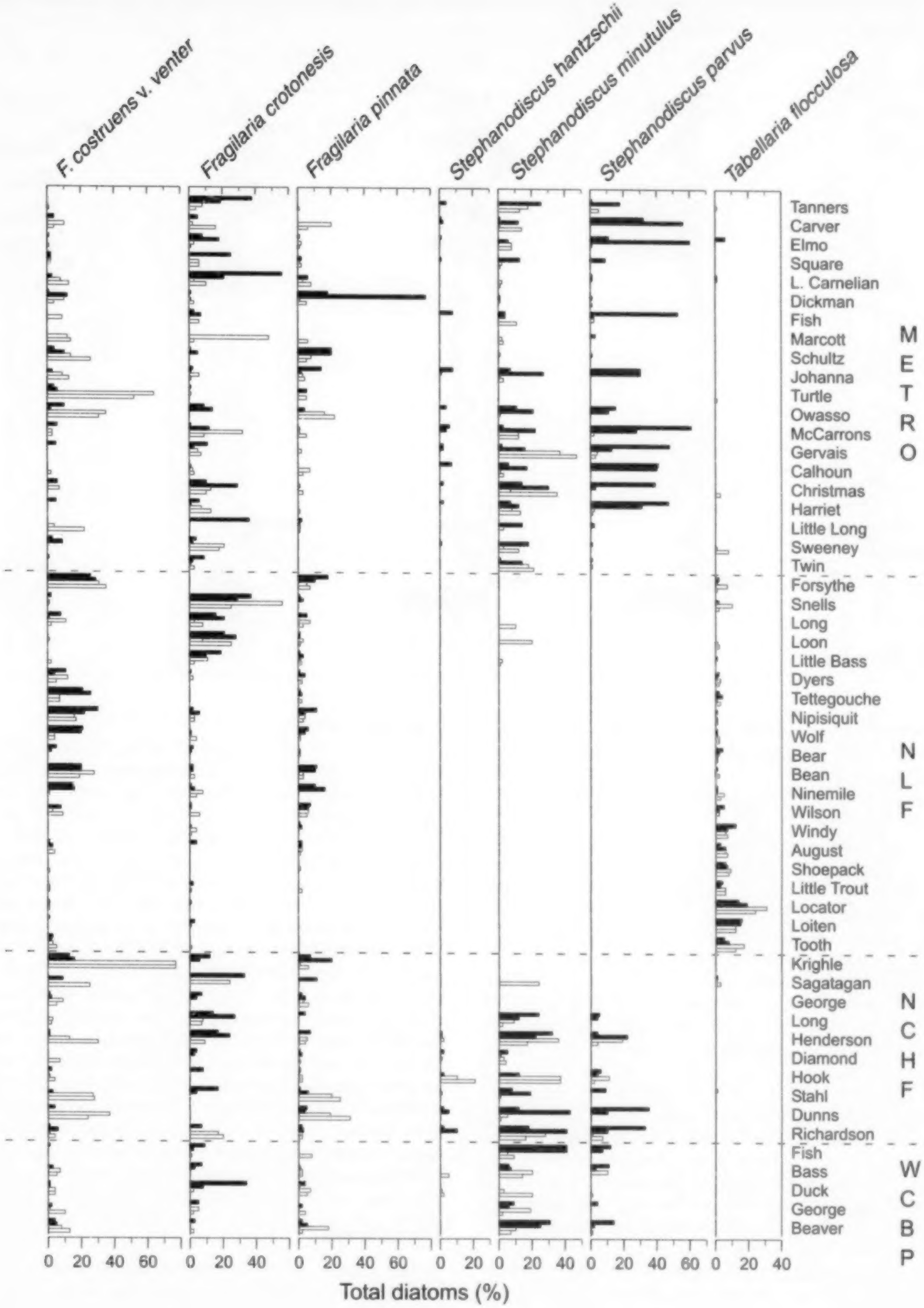
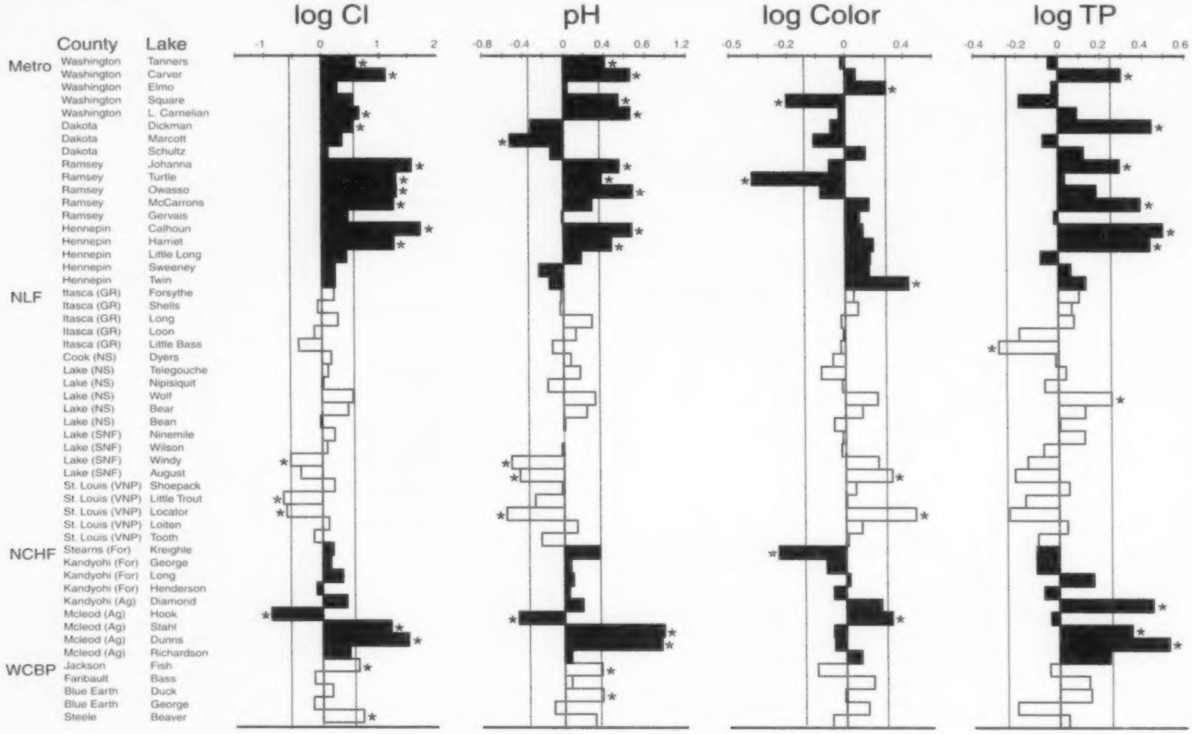


Fig. 3. Diatom-based reconstruction of log Cl, pH, log color, and log TP for 52 of the study lakes. The lakes are grouped by ecoregion, and the results are expressed as the change from 1800 to the present. The lines represent ±RMSEP. Asterisks indicate a change greater than the RMSEP.



most entirely in an undeveloped state (forest and wetland cover types), and although some have been logged historically (and a few partially farmed), the absence of significant land-use conversion is clearly reflected in the absence of water quality change.

Post-1800 water quality changes also appear to be influenced by geomorphic characteristics of the lakes. In the Metro region, Cl and pH increases were greater in larger (surface area) lakes ($r = 0.59$ and 0.56, respectively), which is likely related to the fact that only the smaller lakes in this group retain undeveloped (forested) catchments (Table 2). Among NCHF lakes, those with larger catchment to lake area ratios showed greater increases in TP ($r = 0.67$, $r = 0.03$) and Cl ($r = 0.63$, $r = 0.05$), presumably because larger catchments are more agricultural than smaller ones among this group of lakes ($r = 0.64$). In the WCBP, shallower lakes showed larger TP increases ($r = -0.72$), whereas larger lakes with larger catchments and larger catchment to lake area ratios showed greater increases in pH ($r = 0.65$–0.69); because of small sample size, none of these relationships are significant. Finally, among the relatively pristine NLF lakes, pH changes are negatively correlated with total catchment area ($r = -0.60$, $p = 0.04$). Lakes with smaller catchments tend to be dominated by direct precipitation and have lower buffering capacity and hence may be more susceptible to pH depression from acid rain. Present-day pH and catchment area are correlated ($r = 0.44$, $p = 0.05$) among NLF lakes.

Natural variability

Before 1800, Minnesota had not been settled by Europeans; hence, the anthropogenic influence on lake systems was likely extremely low. Therefore, the difference in water chemistry between 1750 and 1800 is a measure of natural variability. This natural variability is expressed as the change from 1750 to 1800, in log units (Fig. 4), and the regional water chemistry means for these two periods are also compared (Fig. 5). Among the lakes, only two show a change in Cl that exceeds the RMSEP over the 50-year period between 1750 and 1800; lakes in the NLF ecoregion show the least amount of change in Cl concentration (Fig. 4). Only six of the study lakes show a significant change in pH over this time, and there are no clear regional trends. Only one lake records a significant shift in color, and there do not appear to be any regional trends in this variable. Similarly, there are no clear regional trends in TP, and only one lake shows a significant change. In terms of nutrient concentrations, the presettlement range of values for both the Metro and NLF lakes is quite small, in contrast with those in the NCHF and WCBP ecoregions (Fig. 5).

Discussion

The natural environmental gradients that characterize Minnesota are reflected in the diatom assemblages of the individual ecoregions as differences in relative abundances

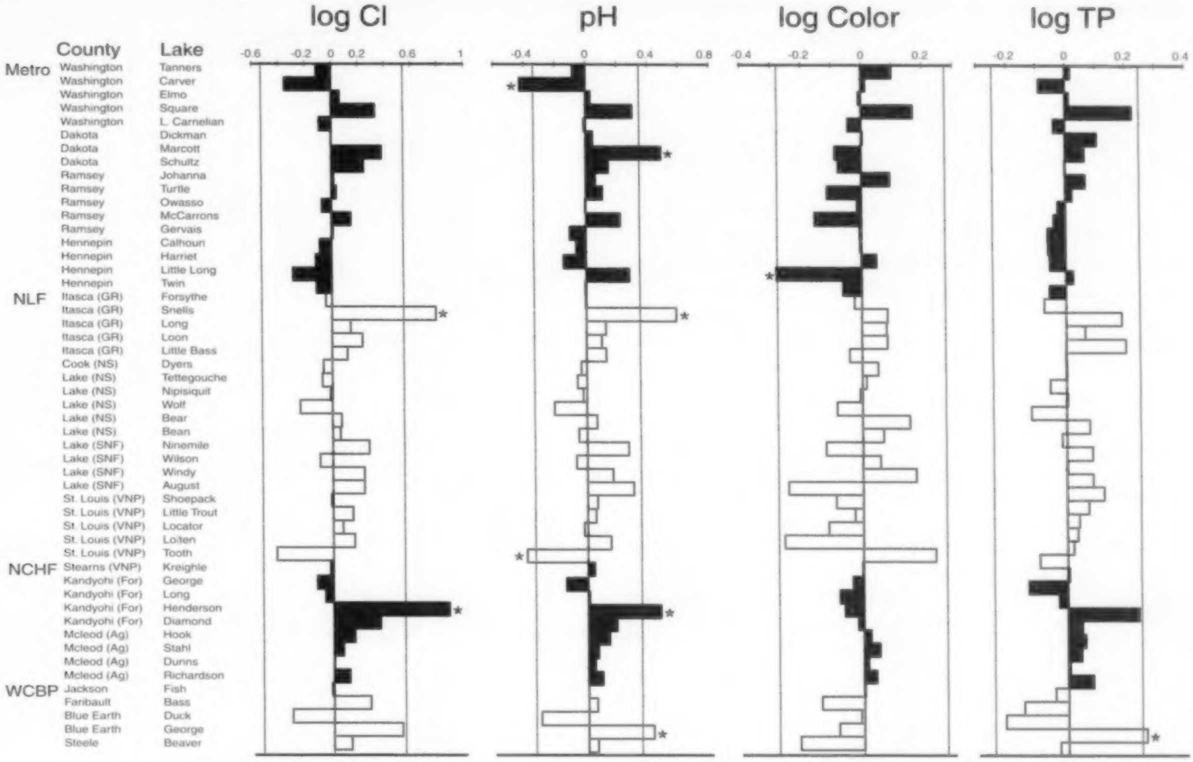


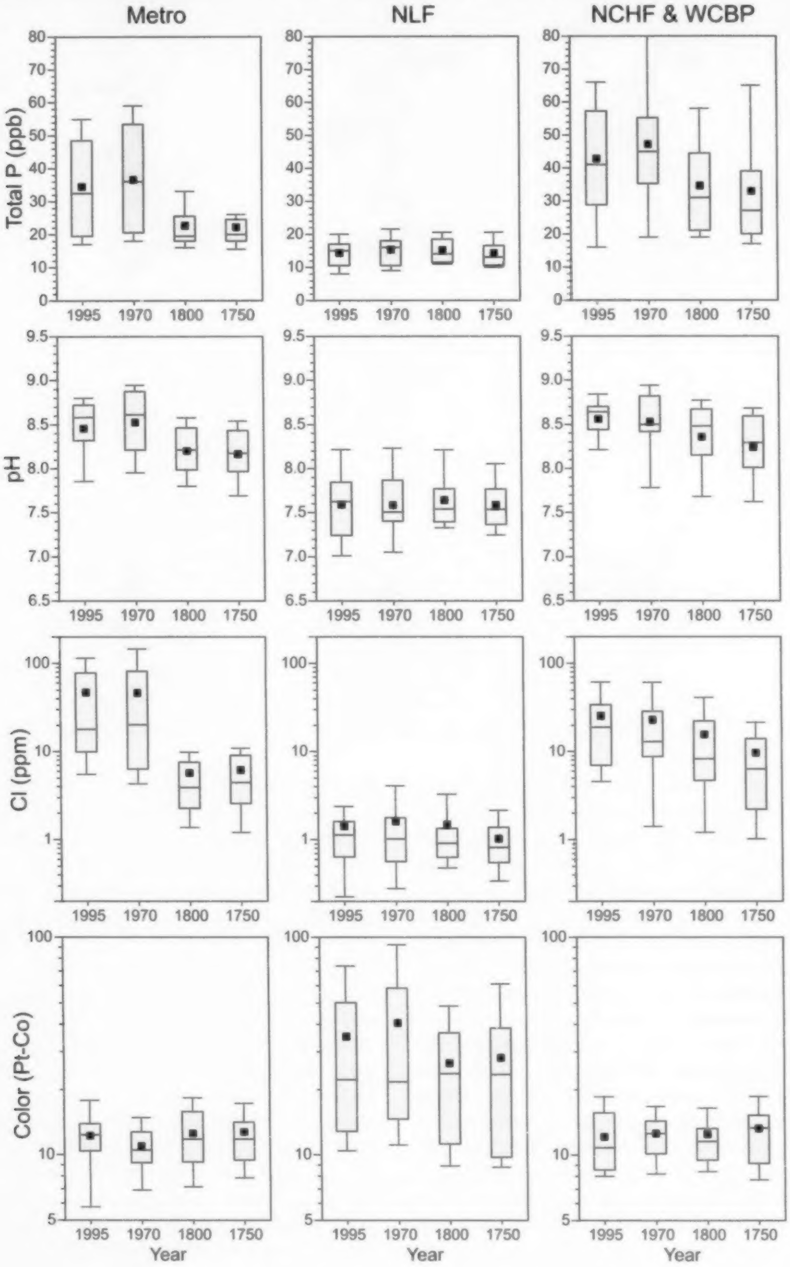
Fig. 4. Diatom-based reconstructions of water quality variables expressed as the change from 1750 to 1800. The lines represent ±RMSEP. Asterisks indicate a change greater than the RMSEP.

among a suite of about a dozen planktonic taxa. The majority of these taxa are found in lakes throughout the state, albeit in varied proportions; however, several species are more restricted in their distribution. These include *Aulacoseira granulate* in the south-central and southwestern part of the region, *Tabellaria flocculosa* and *Cyclotella stelligera* in the NLF region in the northeast, and small *Stephanodiscus* species, which are widespread except in the NLF. Human impacts have not only changed the relative abundance of individual taxa within an ecoregion but in some cases have reduced or nearly eliminated some taxa and expanded the range of others. Thus, *Cyclotella michiganiana* and *Cyclotella pseudostelligera*, which were abundant in the Metro region before settlement, are now largely absent and *Stephanodiscus hantzschii* Grunow *S. parvus* Stoermer & Håkansson has expanded. These floristic changes, which are particularly large in the Metro region, are reflected in the reconstructed water chemistry values for Cl, P, and to some extent pH and color.

Chloride is a conservative ion, and changes in concentration can result from evaporative concentration or dilution driven by differences in precipitation relative to evaporation. However, an increase in Cl also can occur because of the seepage of road salts into lake basins. Little natural variation in Cl occurs in these Minnesota lakes over this time scale, particularly for the NLF and Metro regions. In the NLF ecoregion, precipitation exceeds evaporation, and thus the lakes are not very affected by evaporative concentration. In contrast, in the agricultural ecoregions of NCHF and WCBP, where on average precipitation is lower and evaporation is higher, evaporative enrichment between 1750 and 1800 may have increased ionic concentration. Although these increases are not individually significant, the trend for the group as a whole is (paired t test, $t = 2.16$, $p < 0.05$). Since European settlement, however, prominent and statistically significant changes are evident. All of the lakes in the Metro region, and some in the agricultural regions, have recorded an increase in Cl. The average Cl value for the metro group of lakes has risen by an order of magnitude, a change that is highly significant (paired t test, $t = 7.03$, $p < 0.0001$). It seems likely that this strong directional signal between the modern and presettlement conditions is a result of the seepage of road salts into the lakes and possibly leakage from septic systems in outlying areas. Increased concentrations of road salts have also been observed in lakes in some parts of the northeastern United States (Dixit et al. 1999; Siver et al. 1999).

Regional trends in pH do occur, although they are less distinct than for some other variables. The majority of decreases in pH since settlement are in the NLF ecoregion. These lakes have a low buffering capacity, making them susceptible to acidification by acid rain; however, only three lakes showed a significant decrease in pH since settlement. These results are consistent with conclusions from the

Fig. 5. Distribution by ecoregion of the reconstructed variables for 1750, 1800, 1970, and 1995 (modern) core sections. Variables that were log transformed (Cl, color, and TP) are expressed as back-transformed values. Boxes represent interquartile ranges, bars delineate upper and lower 10%, and the center line is the median. Means are shown by solid squares.



PIRLA project (Paleoecological Investigation of Recent Lake Acidification; Kingston et al. 1990), which included three lakes from the NLF ecoregion and found changes in diatom-inferred pH since 1860 of less than 0.2 pH unit. The one lake in this region that records a significant increase in pH between 1750 and 1800 is situated in more carbonate-rich terrain near the city of Grand Rapids. Increases in pH exceed the RMSEP in nine Metro region lakes and four agricultural region lakes, although the overall change for each region is not statistically significant (paired *t* test, $p = 0.06$ and 0.15, respectively). These pH increases probably reflect higher levels of algal productivity (caused by nutrient enrichment), which can raise pH by photosynthetic uptake of CO_2 (Hodell et al. 1998).

Table 2. Correlations between changes in diatom-inferred water quality variables (1800 to present day) and geomorphic and land-use characteristics of the study lakes.

	Metro (n = 20)				NLF (n = 20)				WCBP (n = 5)				NCHF (n = 10)			
Characteristics	TP	pH	Cl	Color	TP	pH	Cl	Color	TP	pH	Cl	Color	TP	pH	Cl	Color
Catchment area	–0.04	0.11	0.07	0.21	–0.42	**–0.60**	–0.41	**0.51**	0.11	0.68	0.44	–0.69	**0.70**	0.16	0.30	0.56
Lake area	0.22	**0.56**	**0.59**	–0.22	–0.28	–0.30	–0.26	0.04	0.43	0.65	0.19	–0.44	0.40	–0.12	–0.02	0.53
Catchment to lake area ratio	–0.07	–0.05	–0.01	–0.02	–0.30	–0.40	–0.24	0.37	0.04	0.69	0.54	–0.77	**0.67**	0.49	**0.63**	0.20
Lake depth	–0.02	0.33	0.09	0.33	–0.42	–0.10	**–0.47**	–0.02	–0.72	0.11	0.46	–0.52	–0.44	–0.09	–0.09	–0.62
% built up	**0.54**	0.39	**0.71**	–0.10	–0.12	0.30	0.06	–0.01	0.63	0.08	0.13	0.16	–0.26	–0.16	–0.13	–0.10
% road	**0.49**	**0.45**	**0.63**	0.20	–0.11	0.23	0.01	–0.18	–0.08	–0.15	0.22	0.07	–0.12	0.09	0.00	–0.25
% agricultural	–0.42	–0.22	–0.40	0.07	–0.02	0.14	–0.06	–0.07	–0.26	0.17	–0.10	–0.24	**0.72**	0.22	0.28	**0.68**
% forest	–0.35	–0.41	**–0.67**	0.22	0.09	–0.05	0.04	0.01	–0.39	–0.46	0.07	0.21	**–0.62**	–0.08	–0.18	**–0.79**

Note: Water quality changes are expressed as the difference between present day and 1800 reconstructions (log transformed, except pH). Values in bold are statistically significant ($p < 0.05$). Metro, Twin Cities metropolitan area; NLF, Northern Lakes and Forests ecoregion; NCHF, North Central Hardwood Forests ecoregion; WCBP, Western Corn Belt Plains ecoregion (refer to text for description of regions).

Changes in water color (DOC) can result from changes in vegetation and soils (Schindler et al. 1996; Williamson et al. 1999) or photobleaching of DOC (Osburn et al. 2001), induced by either natural or anthropogenic factors. Only eight lakes have changed significantly in color since 1800. The largest postsettlement changes are in the NLF ecoregion, where the coniferous vegetation and peaty soils contribute to regionally high DOC levels. However, the apparent increase in mean regional water color (1800 to the present) is not regionally significant (paired t test, $t = 1.83$, $p = 0.8$), and a similar variability in water color is apparent between 1750 and 1800. Such results imply that modern land-use practices are not significantly altering the DOC content of these lakes and that the majority of change is a result of natural variation in vegetation and climate.

Natural changes in vegetation, soils, and climate can alter nutrient concentrations in lakes (Fritz 1996; Kilham et al. 1996), as can a variety of anthropogenic activities. Only one lake shows a significant change in TP between 1750 and 1800, and no regional trends are apparent. However, very clear trends occur from presettlement to the present, in terms of both mean TP concentrations and the range of variation. There is no directional change in TP since 1800 in the NLF ecoregion, but large increases (exceeding the RMSEP) occur in the other ecoregions. The increase in TP is highly significant in the Metro group of lakes (paired t test, $t = 3.39$, $p < 0.01$) but not so in the agricultural regions (NCHF and WCBP combined: paired t test, $t = 1.29$, $p = 0.22$). The clear trends among ecoregions since settlement (and the lack of those trends from 1750 to 1800) are a strong indication that modern land-use practices have had significant effects on the trophic status of these aquatic systems. Although the NLF ecoregion is the most pristine in this study, there are local impacts such as cottaging (Hall and Smol 1996) and logging, both historic and recent (Fritz et al. 1993), that have the potential to increase P loading to these systems. However, these influences appear to have been relatively minor on average, as there has been very little change in the TP levels of NLF lakes since European settlement.

A noticeable increase in small *Stephanodiscus* species that have high P optima (Bradbury 1975; Fritz et al. 1993; Bennion et al. 1996) occurs in the postsettlement core sections of most Metro region lakes. Although many of these lakes have been the targets of cleanup efforts over the past few decades, most are more nutrient rich today than before settlement. Nonetheless, of the six lakes in the Metro region that show increases in TP since 1800 that exceed the RMSEP (and would be classified today as eutrophic to hypereutrophic), three of these were eutrophic in 1800, based on these reconstructions. This is not to say that cleanup efforts should not continue, but an awareness of baseline conditions for these lakes could be used to better inform management goals for restoration.

By reconstructing water chemistry conditions before European settlement in a suite of lakes in regions with different land-use histories, it is possible to begin to evaluate the amount of change that can be attributed to anthropogenic influences. These data also contribute to an understanding of to what degree the changes reflect natural variability and how much and what types of change are actually related to current land-use practices. Overall, it seems that increases in

Cl and TP in the urban and agricultural regions have been primarily due to human impacts via the seepage of road salts into lake basins and the runoff of nutrients from agricultural and urbanized areas. Indeed, increases in Cl (from presettlement to modern) in Metro region lakes are most strongly correlated with the percentage of watershed area devoted to residential or commercial land use ($r = 0.71$) and road surfaces ($r = 0.63$), whereas TP increases show significant correlation with the percentage of watershed area presently in built-up (residential or commercial) land use ($r = 0.54$). Likewise, TP increases among non-Metro lakes of the NCHF are most strongly correlated with the proportion of watershed devoted to agriculture ($r = 0.72$).

The analysis of presettlement conditions allows recent limnological changes to be viewed in the context of variation over a longer period of time. Although Cl concentration has increased in some urban lakes since 1970 (Ramstack et al. 2003), it is clear that the largest increase predates 1970. Similarly, although TP concentrations have changed in recent decades in lakes in both urban and agricultural areas of the state (Ramstack et al. 2003), a much larger change occurred sometime between regional settlement and 1970.

The data also suggest that in addition to changing average regional conditions, human activities also have affected the range of limnological variation. The range of presettlement variation in TP and Cl appears to be greater in agricultural parts of the state than in the lakes of the Metro region where native vegetation was originally deciduous forest. However, human impacts appear to have increased the range of variation in the urban areas of the Metro region, particularly with respect to nutrients.

In terms of changes in pH, anthropogenic influences may be causing a decline in a small number of particularly sensitive lakes with a naturally low buffering capacity, but on a population basis, these trends are not regionally significant. An increase in pH is evident in some Metro region and agricultural lakes, likely the result of eutrophication. Color is the one variable measured here that does not seem to have been significantly affected by post-European land-use practices, and any fluctuations in water color since settlement appear to be due to natural changes in vegetation and soils within the watersheds.

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Appendix A

Tables A1 and A2 appear on the following pages.

Table A1. Location and water quality characteristics of the study lakes (modified from Engstrom et al. 1999).

Map No.	Ecoregion	County	Lake	Latitude, N	Longitude, W	pH	Color (Pt–Co)	Cl (mg·L^{-1})	TP (mg·L^{-1})
1	Metro	Washington	Tanners	44°57.00′	92°58.88′	8.9	26	99.0	0.056
2	Metro	Washington	Carver	44°54.36′	92°58.83′	8.7	24	123.0	0.036
3	Metro	Washington	Elmo	44°59.37′	92°52.96′	8.5	8	17.0	0.010
4	Metro	Washington	Square	45°09.40′	92°48.26′	8.7	4	5.6	0.012
5	Metro	Washington	L. Carnelian	45°07.07′	92°47.79′	8.8	7	8.3	0.009
6	Metro	Dakota	Dickman	44°51.69′	93°04.74′	7.9	13	58.0	0.105
7	Metro	Dakota	Fish	44°49.35′	93°10.03′	7.6	19	101.0	0.079
8	Metro	Dakota	Marcott	44°48.95′	93°04.02′	7.8	10	36.0	0.019
9	Metro	Dakota	Schultz	44°47.07′	93°07.47′	7.8	18	32.0	0.026
10	Metro	Ramsey	Johanna	45°02.43′	93°09.93′	8.2	17	119.0	0.036
11	Metro	Ramsey	Turtle	45°06.22′	93°08.18′	8.3	8	22.0	0.027
12	Metro	Ramsey	Owasso	45°02.28′	93°07.45′	8.1	15	81.0	0.040
13	Metro	Ramsey	McCarrons	44°59.85′	93°06.67′	8.6	19	74.7	0.048
14	Metro	Ramsey	Gervais	45°01.05′	93°04.30′	8.3	18	102.0	0.033
15	Metro	Hennepin	Calhoun	44°56.63′	93°18.79′	8.7	9	117.0	0.028
16	Metro	Hennepin	Christmas	44°53.85′	93°32.47′	8.9	7	23.0	0.015
17	Metro	Hennepin	Harriet	44°55.15′	93°18.58′	8.9	9	96.0	0.027
18	Metro	Hennepin	Little Long	44°56.88′	93°42.47′	8.8	9	3.7	0.009
19	Metro	Hennepin	Sweeney	44°59.44′	93°20.48′	8.4	18	183.0	0.046
20	Metro	Hennepin	Twin	44°59.52′	93°20.17′	8.5	14	100.0	0.022
21	NLF	Itasca (GR)	Forsythe	47°15.97′	93°36.06′	6.9	85	0.7	0.021
22	NLF	Itasca (GR)	Snells	47°14.46′	93°40.16′	8.2	18	6.3	0.024
23	NLF	Itasca (GR)	Long	47°13.63′	93°39.39′	8.1	10	2.1	0.013
24	NLF	Itasca (GR)	Loon	47°13.95′	93°38.42′	8.6	9	2.8	0.011
25	NLF	Itasca (GR)	Little Bass	47°17.05′	93°36.09′	8.2	17	1.8	0.013
26	NLF	Cook (NS)	Dyers	47°31.62′	90°58.79′	7.6	69	0.8	0.027
27	NLF	Lake (NS)	Tettegouche	47°20.66′	91°16.18′	7.3	33	0.4	0.017
28	NLF	Lake (NS)	Nipisiquit	47°21.31′	91°14.94′	7.4	23	0.3	0.016
29	NLF	Lake (NS)	Wolf	47°22.61′	91°11.58′	7.8	18	1.9	0.014
30	NLF	Lake (NS)	Bear	47°17.05′	91°20.62′	7.5	9	0.4	0.011
31	NLF	Lake (NS)	Bean	47°18.51′	91°18.02′	7.6	8	0.3	0.017
32	NLF	Lake (SNF)	Ninemile	47°34.55′	91°04.88′	7.4	17	0.8	0.017
33	NLF	Lake (SNF)	Wilson	47°40.45′	91°04.53′	7.5	14	0.3	0.013
34	NLF	Lake (SNF)	Windy	47°44.13′	91°04.31′	6.9	98	0.3	0.012
35	NLF	Lake (SNF)	August	47°45.79′	91°36.37′	7.2	97	0.9	0.015
36	NLF	St. Louis (VNP)	Shoepack	48°29.82′	92°53.27′	6.6	117	0.2	0.019
37	NLF	St. Louis (VNP)	Little Trout	48°23.72′	92°31.63′	7.4	3	0.3	0.007
38	NLF	St. Louis (VNP)	Locator	48°32.47′	93°00.34′	7.0	58	0.4	0.009
39	NLF	St. Louis (VNP)	Loiten	48°31.60′	92°55.61′	7.1	29	0.3	0.008
40	NLF	St. Louis (VNP)	Tooth	48°23.88′	92°38.59′	6.8	39	0.4	0.012
41	NCHF	Stearns (For)	Krighle	45°34.72′	94°28.69′	8.5	3	1.2	0.011
42	NCHF	Stearns (For)	Sagatagan	45°34.47′	94°23.45′	8.5	7	3.7	0.027
43	NCHF	Kandyohi (For)	George	45°14.55′	94°59.06′	8.8	5	28.4	0.015

Table A1 (*concluded*).

Map No.	Ecoregion	County	Lake	Latitude, N	Longitude, W	pH	Color (Pt–Co)	Cl ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)
44	NCHF	Kandyohi (For)	Long	45°19.87′	94°51.13′	8.5	9	9.2	0.019
45	NCHF	Kandyohi (For)	Henderson	45°13.82′	94°59.56′	8.6	7	17.6	0.022
46	NCHF	Kandyohi (Ag)	Diamond	45°11.16′	94°51.23′	8.6	16	16.1	0.080
47	NCHF	Mcleod (Ag)	Hook	44°57.21′	94°20.47′	8.4	15	25.3	0.068
48	NCHF	Mcleod (Ag)	Stahl	44°57.18′	94°25.24′	8.4	20	11.6	0.046
49	NCHF	Meeker (Ag)	Dunns	45°09.50′	94°25.74′	8.6	15	17.5	0.139
50	NCHF	Meeker (Ag)	Richardson	45°09.57′	94°26.40′	8.3	18	17.4	0.098
51	WCBP	Jackson	Fish	43°50.82′	95°02.67′	8.8	9	20.2	0.038
52	WCBP	Faribault	Bass	43°49.22′	94°04.67′	9.0	20	15.0	0.081
53	WCBP	Blue Earth	Duck	44°13.07′	93°48.89′	8.9	12	16.2	0.065
54	WCBP	Blue Earth	George	44°14.02′	93°52.29′	9.2	25	11.2	0.130
55	WCBP	Steele	Beaver	43°53.52′	93°20.90′	8.9	9	15.0	0.030

Note: Metro, Twin Cities metropolitan area; NLF, Northern Lakes and Forests ecoregion (GR, Grand Rapids; NS, North Shore; SNF, Superior National Forest; VNP, Voyageurs National Park); NCHF, North Central Hardwood Forests ecoregion (For, watershed is primarily forested; Ag, agriculturally dominated watershed); WCBP, Western Corn Belt Plains ecoregion (refer to text for description of regions).

Table A2. Geomorphic and land-use characteristics of the study lakes (modified from Engstrom et al. 1999).

Map No.	Ecoregion	Lake	Max. depth (m)	Lake area (Ha)	Catchment area (Ha)	Catchment to lake area	% built up	% agriculture	% forest	% road
1	Metro	Tanners	13.7	30.2	669.4	22.2	76.2	0.0	2.0	7.7
2	Metro	Carver	11.0	19.6	864.0	44.1	51.2	17.8	11.5	5.6
3	Metro	Elmo	42.7	114.6	2075.6	18.1	10.0	50.8	11.5	0.9
4	Metro	Square	20.7	81.9	226.0	2.8	10.3	15.0	33.6	1.7
5	Metro	L. Carnelian	19.2	46.7	1853.8	39.7	10.1	17.1	34.0	0.8
6	Metro	Dickman	2.4	9.1	56.9	6.2	33.0	8.9	26.9	1.9
7	Metro	Fish	10.1	12.5	1505.6	120.8	86.1	0.0	1.0	5.3
8	Metro	Marcott	10.1	7.5	251.0	33.6	9.6	30.6	32.6	0.2
9	Metro	Schultz	4.9	5.3	69.1	13.1	1.6	0.0	67.0	0.0
10	Metro	Johanna	12.5	86.4	1025.9	11.9	67.1	0.0	2.9	7.1
11	Metro	Turtle	8.8	133.3	180.3	1.4	46.0	0.0	0.0	1.5
12	Metro	Owasso	12.2	150.9	423.0	2.8	47.5	0.0	2.5	3.0
13	Metro	McCarrons	17.4	30.1	412.1	13.7	57.8	0.0	0.0	3.9
14	Metro	Gervais	12.5	94.4	4028.0	42.7	55.6	0.0	4.3	7.5
15	Metro	Calhoun	27.4	168.5	2515.1	14.9	83.9	0.0	0.8	9.8
16	Metro	Christmas	26.5	105.7	189.3	1.8	54.9	1.3	3.3	2.9
17	Metro	Harriet	25.0	138.6	387.3	2.8	66.7	0.0	6.1	7.8
18	Metro	Little Long	23.2	21.8	32.4	1.5	0.0	10.7	34.6	1.2
19	Metro	Sweeney	7.6	28.3	1010.9	35.7	64.7	0.0	6.6	1.2

Table A2 (*concluded*).

Map No.	Ecoregion	Lake	Max. depth (m)	Lake area (Ha)	Catchment area (Ha)	Catchment to lake area	% built up	% agriculture	% forest	% road
20	Metro	Twin	17.1	8.7	33.5	3.8	28.4	0.0	46.3	4.3
21	NLF	Forsythe	3.1	27.1	191.2	7.1	0.0	4.5	44.1	0.8
22	NLF	Snells	15.2	35.8	288.2	8.1	0.0	38.6	44.2	1.1
23	NLF	Long	22.9	54.5	164.7	3.0	0.1	6.4	54.3	1.0
24	NLF	Loon	21.0	92.9	363.6	3.9	4.4	13.0	38.4	1.3
25	NLF	Little Bass	18.9	64.5	540.1	8.4	4.2	9.8	54.6	0.7
26	NLF	Dyers	6.1	27.8	854.2	30.7	0.0	0.0	89.3	0.6
27	NLF	Tettegouche	4.6	26.7	102.7	3.8	0.0	0.0	76.6	0.0
28	NLF	Nipisiquit	5.5	23.7	297.7	12.6	0.0	0.0	71.4	0.0
29	NLF	Wolf	7.3	13.1	67.9	5.2	4.7	0.0	77.0	0.0
30	NLF	Bear	8.8	18.3	60.0	3.3	0.0	0.0	76.6	0.0
31	NLF	Bean	7.9	12.5	64.3	5.1	0.0	0.0	73.1	0.0
32	NLF	Ninemile	9.1	120.3	209.0	1.7	0.0	0.0	63.5	0.3
33	NLF	Wilson	14.9	257.1	718.9	2.8	0.0	0.0	61.3	0.0
34	NLF	Windy	11.9	184.6	1705.0	9.2	0.0	0.0	69.1	0.3
35	NLF	August	5.8	76.5	936.8	12.3	0.0	0.0	76.2	0.3
36	NLF	Shoepack	7.3	155.4	1767.9	11.4	0.0	0.0	68.2	0.0
37	NLF	Little Trout	29.0	107.6	139.8	1.3	0.0	0.0	56.6	0.0
38	NLF	Locator	15.9	54.1	1489.4	27.5	0.0	0.0	81.1	0.0
39	NLF	Loiten	14.9	39.0	241.7	6.2	0.0	0.0	80.8	0.0
40	NLF	Tooth	13.1	23.6	151.0	6.4	0.0	0.0	78.0	0.0
41	NCHF	Kreighle	17.1	41.0	87.8	2.1	0.0	15.7	48.6	1.1
42	NCHF	Sagatagan	14.3	88.7	251.8	2.8	1.7	16.3	43.4	0.7
43	NCHF	George	9.1	91.6	102.2	1.1	21.0	1.9	17.3	0.6
44	NCHF	Long	13.4	129.8	362.7	2.8	12.0	26.0	24.1	0.8
45	NCHF	Henderson	12.2	29.1	59.4	2.0	24.0	18.5	7.7	3.4
46	NCHF	Diamond	8.2	644.8	3589.7	5.6	4.5	56.4	4.3	0.9
47	NCHF	Hook	5.5	133.1	620.1	4.7	3.3	61.0	4.3	0.5
48	NCHF	Stahl	10.7	54.6	629.6	11.5	0.0	55.6	11.0	1.2
49	NCHF	Dunns	6.1	63.0	1381.3	21.9	7.7	50.2	11.2	1.2
50	NCHF	Richardson	14.3	48.3	1168.5	24.2	2.4	73.1	4.1	1.0
51	WCBP	Fish	8.2	120.7	649.4	5.4	1.5	76.0	3.2	1.0
52	WCBP	Bass	6.1	75.7	136.5	1.8	9.5	44.8	6.8	1.0
53	WCBP	Duck	7.6	116.6	385.0	3.3	3.0	66.2	1.3	1.2
54	WCBP	George	8.5	36.2	54.4	1.5	0.0	48.8	11.1	1.1
55	WCBP	Beaver	8.2	37.8	71.1	1.9	9.5	39.7	15.6	1.6

Note: Catchment and catchment to lake area ratios for total terrestrial catchment, and land-use cover types as a percentage of the primary catchment (which excludes areas drained by upstream lakes, see text). Metro, Twin Cities metropolitan area; NLF, Northern Lakes and Forests ecoregion; NCHF, North Central Hardwood Forests ecoregion; WCBP, Western Corn Belt Plains ecoregion (refer to text for description of regions). Max., maximum.

Differences in juvenile phenotypes and survival between hatchery stocks and a natural population provide evidence for modified selection due to captive breeding

Kathryn E. Kostow

Abstract: Juvenile phenotypes and fitness as indicated by survival were compared for naturally produced steelhead (*Oncorhynchus mykiss*), a new local hatchery stock, and an old nonlocal hatchery stock on the Hood River, Oregon, U.S.A. Although the new hatchery stock and the naturally produced fish came from the same parent gene pool, they differed significantly at every phenotype measured except saltwater age. The characteristics of the new hatchery stock were similar to those of the old hatchery stock. Most of the phenotypic differences were probably environmentally caused. Although such character changes would not be inherited, they may influence the relative fitness of the hatchery and natural fish when they are in the same environment, as selection responds to phenotypic distributions. A difference in fitness between the new hatchery stock and naturally produced fish was indicated by significant survival differences. Acclimation of the new hatchery stock in a "seminatural" pond before release was associated with a further decrease in relative smolt-to-adult survival with little increase in phenotypic similarity between the natural and hatchery fish. These results suggest that modified selection begins immediately in the first generation of a new hatchery stock and may provide a mechanism for genetic change.

Résumé : Les phénotypes des jeunes et leur survie (comme mesure de fitness) ont pu être comparés chez des truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) nées en nature, des truites d'un nouveau stock local de pisciculture et des truites d'un ancien stock non local de pisciculture dans la rivière Hood, Oregon, É.-U. Bien que le nouveau stock de pisciculture et le stock naturel proviennent du même pool génétique parental, ils diffèrent significativement par tous les caractères phénotypiques mesurés, sauf l'âge en eau salée. Les caractéristiques du nouveau stock de pisciculture ressemblent à celles de l'ancien stock de pisciculture. La plupart des différences phénotypiques sont probablement attribuables à l'environnement. Bien que de tels caractères ne soient pas transmis génétiquement, ils peuvent affecter le fitness relatif des poissons de pisciculture et des poissons sauvages quand ils cohabitent dans le même milieu, puisque la sélection réagit à la distribution des phénotypes. Il existe des différences significatives de survie, donc de fitness, entre le nouveau stock de pisciculture et le stock sauvage. L'acclimatation du nouveau stock de pisciculture dans un étang « semi-naturel » avant sa libération en nature entraîne une diminution additionnelle de la survie des saumoneaux jusqu'au stade adulte et augmente peu la ressemblance phénotypique entre les poissons de pisciculture et les poissons sauvages. Ces résultats indiquent que la sélection modifiée agit immédiatement dès la première génération dans un nouveau stock de pisciculture et qu'elle peut fournir un mécanisme pour le changement génétique.

[Traduit par la Rédaction]

Introduction

Phenotypic and fitness differences between wild fish and hatchery fish that have been captive for several generations are well documented (e.g., Reisenbichler and McIntyre 1977; Fleming et al. 1996; Johnsson et al. 2001). Many of the differences are argued by the authors to have a genetic basis (e.g., Fleming et al. 1994; Berejikian 1995; Kallio-Nyberg and Koljonen 1997). The mechanisms that have been proposed to cause genetic divergence between hatchery and wild fish include modified selection pressures caused by different survivals and mortalities in the artificial versus natural environments (Reisenbichler and Rubin 1999; Ford 2002). Other mechanisms include accumulation of genetic load caused by relaxed selection during captivity (Lynch and O'Hely 2001) and genetic changes caused by small broodstocks and other demographics that lower effective population sizes (Ryman and Laikre 1991; Ryman et al. 1995). These mechanisms may work concurrently to eventually erode the fitness of a captive population when it is returned to the wild (Woodworth et al. 2002).

The idea that modified selection pressures might cause hatchery and wild fish to diverge has been criticized by some resource managers in the northwestern United States because the survival of fish while in captivity can be very high, up to 90% for fish reared from eggs to smolts (ODFW and USFWS 1996). Given these high survivals, some managers argue that there appears to be little opportunity for se-

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lection to occur either for or against particular individuals while they are captive (see, for example, the requirements for domestication selection presented by Doyle (1983)). However, it may be possible for modified selection to continue to affect the hatchery fish after they are released. In order for this to occur, the hatchery fish would have to continue to experience different selection pressures after release than those experienced by the wild fish. One way this might occur is if the hatchery and wild fish express significantly different phenotypes while they are in the same natural environment and experience different survivals under the same conditions.

It is a common practice in steelhead (*Oncorhynchus mykiss*) hatchery programs to accelerate growth rates and smoltification to produce large, yearling smolts (see examples of release criteria in ODFW and USFWS (1996)). Managers adopt this practice in an effort to optimize postrelease survival of the hatchery fish (Wagner et al. 1963; Wagner 1967) while reducing or eliminating residual hatchery fish (Jonasson et al. 1995; Bigelow 1995). It is generally recognized that this protocol could make hatchery steelhead juveniles larger and younger than wild fish (Berejikian et al. 1996). Many chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) hatchery programs also increase growth rate and size at release to produce vigorous smolts and to improve smolt-to-adult survival (Mahnken et al. 1982; Dickhoff et al. 1995).

The implications of intentionally manipulating juvenile phenotypes in a hatchery program are particularly important when the program is implemented for the purpose of increasing the size of a wild population. The current management perspective is that hatchery fish in a conservation hatchery program of this kind should be as "similar" to wild fish as possible to lower both genetic and ecological risks to wild fish (Independent Multidisciplinary Science Team 2000; McMichael et al. 2000). "Similarity" is most often viewed in a genetic or phenotypic context. One idea associated with this perspective is that if hatchery and wild fish were phenotypically "similar", then selection in the natural environment might affect the two groups in a "similar" way, decreasing the opportunity for genetic divergence.

However, manipulation of juvenile phenotypes is used in steelhead hatchery programs in the northwestern United States even when the program goal is to produce hatchery fish for conservation purposes with characteristics that are "similar" to those of wild fish (Phillips et al. 2000). Many managers propose that the environmentally caused differences that result from the manipulations are not genetically important because they are not inherited (Swain et al. 1991). However, selection differentials respond to phenotypic distributions and influence fitness regardless of the source of phenotypic variation. After the hatchery fish are released, they are exposed to the same natural environment as wild fish. If the hatchery and wild fish have substantially different phenotypes when they are in the same environment, natural selection may affect them differently. These different selection regimes could lead to eventual genetic divergence between the groups.

A comparison of wild fish that have never been in captivity and hatchery fish from the same parent gene pool may reveal phenotypic and fitness differences that provide the opportunity for modified selection pressures to occur. In this paper, I compare several juvenile phenotypes and fitness as indicated by survival of naturally produced steelhead and hatchery steelhead from a newly founded conservation hatchery program. The evaluation site was the Hood River, a tributary of the lower Columbia River at river kilometre (rkm) 270 in Oregon, U.S.A., which has indigenous winter and summer steelhead populations. Juvenile out-migrants were evaluated over 5 years, from 1994 through 1998. During the evaluation period, two hatchery stocks were released into the Hood River. The "new" hatchery stock was founded starting in 1991 from wild winter steelhead adults captured at Powerdale Dam at rkm 6.4 on the mainstem Hood River. This hatchery program was implemented for the purpose of rapidly increasing the size of the wild winter steelhead population. The "old" hatchery program was implemented to provide a sports fishery in the Hood River and used a nonlocal summer steelhead hatchery stock that was founded in the 1950s.

One may expect significant phenotypic and fitness differences between the old hatchery stock and the native Hood River steelhead populations. The primary question for this paper was how hatchery juveniles from the new hatchery stock compared with naturally produced juveniles that had never been in captivity but came from the same parent gene pool as the hatchery fish. Fish from the new hatchery stock were also compared with fish from the old hatchery stock.

Materials and methods

Study populations

All adult steelhead have been captured and sorted at a trap at Powerdale Dam since 1992. The adult trap captured 100% of the adult steelhead destined to spawn in the Hood River and was located below 100% of the natural production area in the basin. The population sizes and composition of the naturally spawning parent populations that produced the out-migrants in 1994 through 1998 were therefore known. Broodstock for the new hatchery program was collected at the same trap, whereas the old hatchery stock smolts were imported from a different basin.

The new hatchery stock, Hood River Winter Stock, was founded starting in 1991 from wild Hood River winter steelhead. Wild adults were distinguished from any hatchery fish that entered the trap by a lack of fin clips or other marks. Each year of this study, 10–25% of the wild population was taken for the broodstock, sampled throughout the wild run time distribution. The juvenile out-migrants in this study were produced by the 1993 through 1997 brood years. The broodstocks ranged in size from 43 to 82 fish and included from 0% to 48% hatchery fish. The hatchery fish that were used in the broodstock were adult returns from the first years' releases of the same stock. The hatchery protocols used to rear, acclimate, and release the new hatchery stock were those designed for the regular hatchery program, described in Olsen and French (2000) and O'Toole (1991), since the intent was to evaluate the products of the program as it was intended for normal implementation. The hatchery smolts were released directly into the East Fork of the Hood River in 1994 and 1995 but were acclimated for several weeks before release in 1996 through 1998 in a pond that was supplied with some "enriched environmental features" (Flagg and Nash 1999). One hundred percent of the new hatchery stock juveniles were marked with fin and mandible clips.

The old hatchery stock, South Santiam Hatchery Skamania Summer Stock, originated from several tributaries in southwest Washington in the late 1950s (Reisenbichler et al. 1992). The broodstock was transferred to South Santiam Hatchery in Oregon in the 1970s and was released annually into several Oregon rivers, including Hood River, to provide a sports fishery. The total broodstock size during the 1990s was quite large, but only a small part of the production was released into the Hood. The number of parents that produced fish for the Hood ranged from 54 to 92 fish and was composed of 100% hatchery fish. Juveniles from the old hatchery stock were reared outside the Hood Basin then released as smolts directly into the West Fork of the Hood River. One hundred percent of the old hatchery stock juveniles were marked with fin clips.

The two wild steelhead populations in the Hood River were distinguished by adult run time and geographic spawning distribution. The winter steelhead population passed Powerdale Dam between January and June and immediately spawned in the East and Middle forks of the Hood River. The summer steelhead population passed Powerdale Dam between April and October and spawned the following spring in the West Fork of the Hood River, above a partial passage barrier that blocked winter steelhead. Resident rainbow trout were sympatric with the summer steelhead population (Kostow 1995).

Naturally produced juveniles ranged in age from 0 to 4 years so the 1994 through 1998 out-migrants were attributed to past parent-years proportional to their age distributions. The naturally spawning winter steelhead parent population ranged in size from 166 to 491 fish. No winter steelhead hatchery fish were intentionally passed above the dam in brood years 1992 through 1995. However, because of the presence of older smolts that had been produced from earlier brood years and the accidental release of a few adult hatchery fish above the dam, it was determined that 2–3% of the parents of the 1994 through 1996 out-migrants had been hatchery fish from other hatchery stocks outside the Hood Basin. Some hatchery adults from the new winter steelhead hatchery stock were intentionally passed above the dam starting in 1996. The 1997 and 1998 naturally produced out-migrants had 43% and 51% hatchery parents, respectively. Hatchery summer steelhead adults from the South Santiam/Skamania stock were passed above the dam during the study and potentially mingled with the wild summer steelhead in the West Fork. The naturally spawning summer steelhead parent population ranged in size from 652 to 2211 fish but included a much larger hatchery component, 78–88% hatchery fish.

Juvenile phenotype data

Juvenile phenotypes of old hatchery stock juveniles, new hatchery stock juveniles, and naturally produced juveniles were measured during 5 years of juvenile out-migration from 1994 through 1998. The new hatchery stock juveniles were measured for length and weight before release as yearling smolts. Releases occurred between 1 April and 15 May each year. The old hatchery stock juveniles were released as yearling smolts in April. A subsample of juveniles from both hatchery stocks were recaptured along with naturally produced juveniles in four rotary-screw (smolt) traps located in the West Fork at rkm 6.4, in the Middle Fork at rkm 2.1, in the East Fork at rkm 1.6, and in the lower mainstem Hood River at rkm 7.2. The traps were operated from about the first of April to about the end of October each year and were checked on most days. Further details about the field sampling protocols are described in Olsen (2003).

The origin of the juveniles captured in the smolt traps was identified as new hatchery stock, old hatchery stock, or naturally produced fish based on the fin and mandible marks or lack of marks. Phenotype data for the naturally produced winter and summer steelhead were combined, as individuals from these life histories could not be differentiated in the mainstem trap. The fish were measured for length (millimetres), weight (grams), date of capture, and age at capture determined from a juvenile scale sample. Juveniles passing the mainstem smolt trap were moving below all-natural rearing habitats in the basin and were believed to be leaving the Hood River, whereas those captured in the tributary smolt traps may have been moving within the basin. Therefore, the date of capture and the age at capture in the mainstem trap were considered to be the date of and age at out-migration from the Hood Basin. Out-migration from the Hood did not necessarily mean that the fish were smolting and moving into the ocean. Further phenotypic data were collected when the out-migrants returned as adults to the Powerdale trap in 1995 through 2002. Freshwater age, also considered to be the age at smolting, was determined by a saltwater entry mark on the adult scales (Borgerson 1992). Saltwater age and total age at spawning were also determined from the adult scales.

The dates of capture at the mainstem trap (dates of out-migration) and age data from adult scales were combined for all years within origin group: naturally produced, new hatchery stock, and old hatchery stock. However, the mean values and the distributions of the length and weight traits differed within the three origin groups depending on when and where they were measured; therefore, the data were distributed among several treatments. For naturally produced juveniles, two treatment groups were identified: captured in the tributaries (all three traps) and captured in the mainstem trap with data for all years combined. For the old hatchery stock, there were also two treatment groups: captured in the tributary trap (West Fork) and captured in the mainstem trap with data for all years combined. For the new hatchery stock, five treatment groups were analyzed to take into consideration the changes in release strategy part way through the study. For the two years of direct releases (1994–1995), there were two treatments: at release and captured in the mainstem trap. For the three years of acclimated releases (1996–1998), there were three treatments: at release, captured in the tributary trap (East Fork), and captured in the mainstem trap.

Over the 5 years, nearly 3000 juvenile fish were measured in the tributary traps, whereas over 5250 were measured in the mainstem trap. Most of the phenotype sample sizes were therefore large and most of the data sets were approximately normally distributed. Exceptional data sets included the date of out-migration for naturally produced juveniles and the length and weight measurements for naturally produced juveniles in the tributaries, all of which had bimodal distributions. The length and weight data were analyzed among all treatment groups using pairwise F tests to compare phenotypic variances and pairwise t tests assuming either equal or unequal variances depending on the results of the F tests to compare mean phenotype values. Dates of out-migration were also compared by applying pairwise t tests and F tests to the dates of capture at the mainstem trap. Freshwater age (interpreted as age at smolting) and saltwater age were compared using t tests.

Further information about the behavior of the naturally produced fish was qualitatively described from the trapping data. Comparisons of age at capture and dates of capture in the mainstem and tributary smolt traps and comparisons of age at capture in the smolt traps and age at smolting determined from adult scales revealed life history complexity among the naturally produced fish that was not present among any hatchery treatments. Graphics were developed to demonstrate and compare the distribution of phenotypic variation for some of the more complex traits and the behavior patterns were described. The only comparison that could be made was that the life history complexity was present in the naturally produced fish but absent in the hatchery fish.

Survival data

The following measures of survival were made as fitness indicators: egg-to-smolt survival, smolt-to-adult survival, egg-to-adult survival, parent per capita production of smolt offspring, and parent per capita production of adult offspring. Survival data were measured for each of the 5 out-migrant years from 1994 through 1998 for naturally produced winter steelhead, naturally produced summer steelhead, the new hatchery stock and the old hatchery stock.

For the two hatchery stocks, the survival data were computed from hatchery monitoring records, smolt release data, and adult return data. The parent populations were the broodstocks in brood years 1993 through 1997 that produced the smolts that were released into the Hood Basin. Egg production was determined from the number of females spawned and the average fecundities of the females. Egg-to-smolt survivals occurred during captivity for the hatchery stocks and were tracked while the fish were in the hatcheries from green eggs to smolt release. Only natural mortalities were considered; losses resulting from intentional culling were not counted. Smolt-to-adult survivals were determined for each out-migrant year from the number of smolts released into the Hood River and the number of adults returning for their first spawning measured at the mouth of the Hood River. Fish from the 1994 through 1998 release years returned for their first spawning from 1995 through 2002. The number of adults at the mouth of the Hood River was determined by the Powerdale Dam counts, where the adults were also aged and assigned to their out-migration year, and the annual harvest rate in the basin below the dam.

The number of naturally spawning parents that produced each out-migrant year was estimated from the juvenile age distribution and counts of adults past Powerdale Dam, corrected for a prespawning mortality of 5% for winter steelhead and 10% for summer steelhead. Naturally spawning wild and hatchery fish were treated equally. Natural egg production was calculated from the number of females passed and an average fecundity of 3500 eggs per female. The egg-to-smolt and smolt-to-adult survivals of naturally produced winter and summer steelhead depended on an estimate of wild smolt production from the Hood River. An estimate of total smolt production from the basin was made from sampling of unmarked juveniles at the mainstem trap. The total production estimate was distributed among summer and winter steelhead using the data from the West Fork trap (summer steelhead) and the traps in the East and Middle forks (winter steelhead). Since it was evident from the size and age data that some of the out-migrants may not have been smolts, only fish larger than 165 mm were used to make the smolt production estimates. Trap efficiencies were regularly monitored and adjusted seasonally to account for variations in flows. A pooled Petersen mark–recapture estimate with Chapman's modification (Ricker 1975) was used to make the juvenile abundance estimates. Further details about the methods used to calculate the number of naturally produced winter and summer smolts are provided in Olsen (2003). The number of adult offspring was determined using the same method that was used for hatchery fish. Fish from the 1994 through 1998 out-migrants returned for their first year of spawning in 1995 through 2002. Age and lower Hood River sports harvest were again taken into consideration to calculate the adult returns to the river mouth for each out-migrant year.

The survival statistics, egg-to-smolt survival, smolt-to-adult survival, egg-to-adult survival, parent per capita production of smolt offspring, and parent per capita production of adult offspring, were computed for each of the out-migrant years for naturally produced winter steelhead, naturally produced summer steelhead, the new hatchery stock, and the old hatchery stock. The results averaged over the 5 years for each group were then compared using pairwise t tests. Within the new hatchery stock group, the two direct-release years (1994–1995) and the three acclimated-release years (1996–1998) were also broken out for comparison to take into consideration the change in release strategy that occurred during the study.

Results

Length and weight of juveniles

Naturally produced juveniles were significantly smaller ($P < 0.001$) than all treatments of both the new and the old hatchery fish stocks measured both as length and as weight (Table 1). They were also more variable in length than hatchery fish, while they showed similar amounts of variation in weight, although in different distributions. Both the length and weight trait distributions of naturally produced fish measured in the tributaries were strongly bimodal (Fig. 1) and the fish were significantly smaller ($P < 0.001$) than when measured in the mainstem trap.

The fish from the new hatchery stock differed in size depending on when and where the fish were measured. The new hatchery stock fish that were directly released were significantly bigger, both in length and weight and both at release and at recapture, than those that were acclimated ($P <$

Table 1. Comparison of juvenile lengths and weights.

Group	*N* (lengths)	Average length (SD) (mm)	*N* (weights)	Average weight (SD) (g)
Natural (tributary)	2289	119.3 (48.5)	1690	28.4 (26.3)
Natural (mainstem)	919	171.3 (29.0)	722	53.6 (23.0)
New hatchery stock (direct release)	915	192.3 (22.8)	915	81.7 (27.9)
New hatchery stock (direct, mainstem)	391	208.0a (15.1)	382	89.3b (22.0e)
New hatchery stock (acclimated release)	1007	185.7 (24.6)	1007	75.2 (28.4)
New hatchery stock (acclimated, tributary)	413	196.6 (16.4c)	383	78.9 (19.9d)
New hatchery stock (acclimated, mainstem)	2535	199.4 (16.6c)	2437	78.0 (20.0d)
Old hatchery stock (tributary)	211	208.5a (21.1)	205	87.0b (26.5)
Old hatchery stock (mainstem)	1371	208.1a (18.0)	1345	88.1b (22.4e)

Note: The lengths of the old hatchery stock measured in the tributaries and in the mainstem were not significantly different from each other or from those of the new hatchery stock at the mainstem trap when the smolts were direct released (indicated by "a"). All other pairwise mean lengths analyzed with *t* tests were significant at $P < 0.001$. The mean weights of the two old hatchery stock samples and the direct-release new hatchery stock sample at the mainstem trap (indicated by "b") were not significantly different from each other. All other pairwise mean weights analyzed with *t* tests were significant at $P < 0.001$. Variances for two of the new hatchery stock lengths and weights (indicated by "c" and "d", respectively) and for the direct-release new hatchery stock and old hatchery stock at the mainstem trap (indicated by "e") were not significantly different. All other pairwise variances analyzed with *F* tests were significantly different at $P < 0.001$.

0.001). Within both the direct-release and the acclimated groups of the new hatchery stock, the fish became significantly longer and heavier as they progressed from release to recapture in the mainstem trap ($P < 0.001$). The change in size was partially due to a loss of the smallest hatchery fish between release and recapture, although other changes in the distribution also occurred (Fig. 2*a*).

The old hatchery stock juveniles were significantly larger ($P < 0.001$) than four of the treatments of the new hatchery stock juveniles measured by both length and weight, but the two stocks were not significantly different in either measurement at the mainstem trap when the new hatchery stock fish were directly released. The average size of old hatchery stock juveniles was uniform from release to out-migration in that neither length nor weight varied by sampling site. Even though the acclimated fish from the new hatchery stock were significantly smaller than fish from the old hatchery stock, they had a size distribution at the mainstem trap that was more similar to that of the old hatchery stock than to that of the naturally produced fish (Fig. 2*b*).

Age at smolting and age at first spawning

Fish from both hatchery stocks were significantly younger when they smolted, as determined by the age at which they entered salt water, than the naturally produced fish ($P < 0.001$). The hatchery fish were also nearly uniform in age, whereas the naturally produced fish were highly variable (Table 2). The younger and lesser variation in freshwater age of the hatchery fish corresponded to younger and lesser variation in total adult age at first spawning. Another consequence of a younger total age was that the average generation time of the hatchery fish was shortened by 1 year, from 4 years for the naturally produced fish to 3 years for both hatchery stocks. The new and old hatchery stocks had nearly identical age distributions. Both stocks were intended to be yearling smolts and this objective was achieved in over 99% of the fish based on ages from adult scales. Both hatchery stocks showed similar characteristics and diversity in saltwater age and were similar to wild fish in this trait.

Time of out-migration

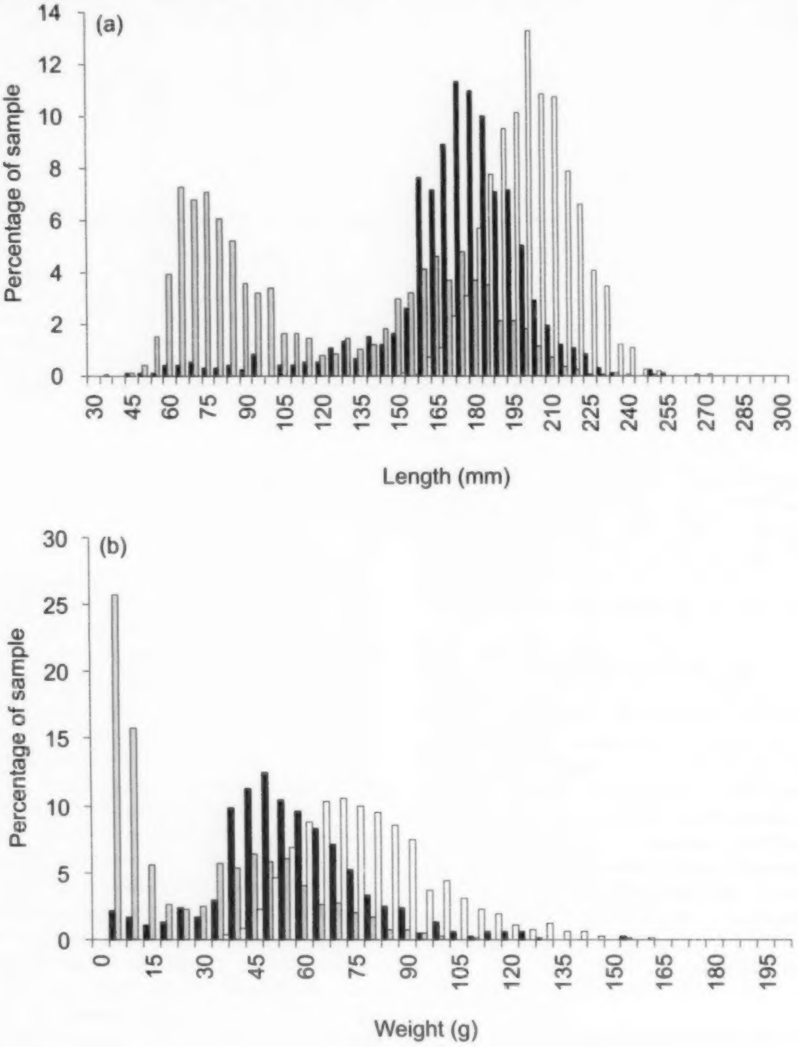
Time of out-migration was measured as the date at which fish were captured in the mainstem trap. This was a difficult trait to measure in this study with a complete lack of bias because two protocols affected sampling, which affected the character of the trait. First, hatchery fish could not out-migrate before they were released. Second, the Hood could not be sampled for out-migrating juveniles all year because the river experiences high flashy flows in the winter and smolt traps cannot safely operate.

Juveniles from both hatchery stocks out-migrated significantly earlier than naturally produced juveniles based on mean date of out-migration ($P < 0.001$). However, the primary difference between the groups was that the out-migration time distribution for natural fish was highly variable, whereas the out-migration time for hatchery fish was very narrow (variance for wild fish (σ_W) = 3377 and variance for hatchery stock (σ_H) = 317, $P < 0.001$, also compared in Fig. 3). A large number of naturally produced fish out-migrated during the spring and the new hatchery stock matched this peak time reasonably well. But less than 1% of either hatchery stock was captured in any smolt trap after the end of June. In contrast, naturally produced fish were captured throughout the season. Their lowest period of movement was during midsummer when river flows were at the lowest levels, and then out-migration increased again in September. Substantial numbers of fish continued to out-migrate through October and it is possible that some continued to out-migrate through the winter after sampling stopped.

Life history complexity

The size, age, and movement data also revealed information about natural juvenile behavior and life history patterns in the Hood Basin that were absent among the hatchery fish. Among the naturally produced fish, small age-0 and age-1

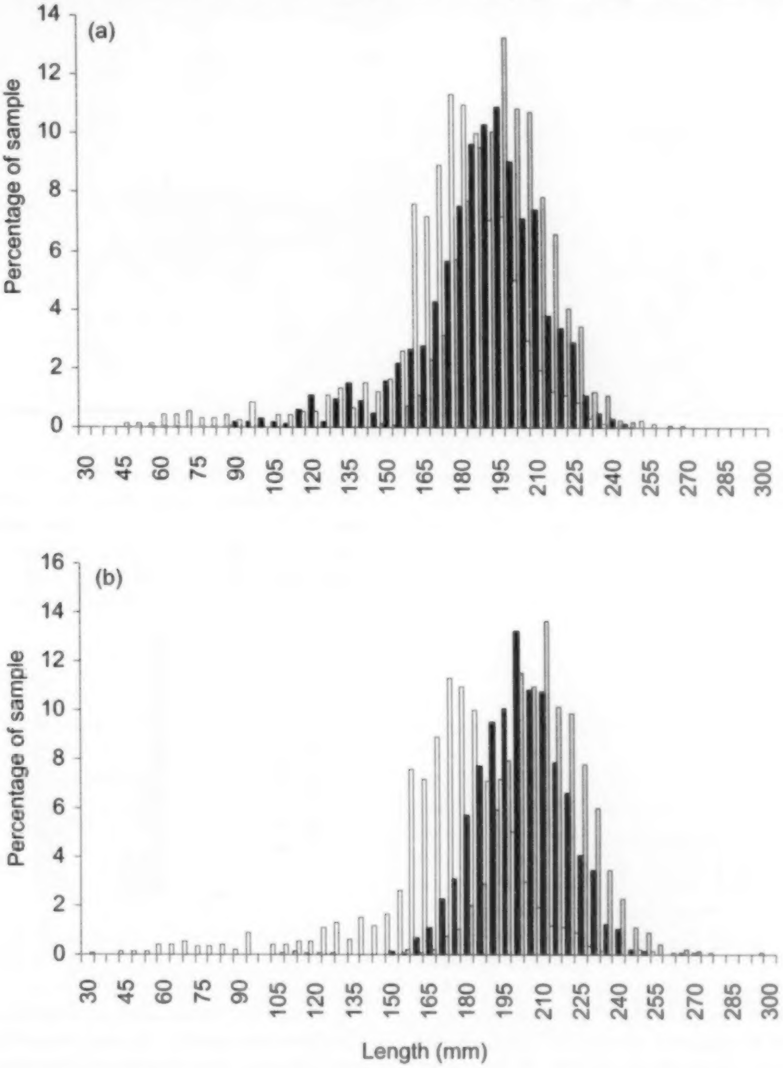
Fig. 1. (*a*) Length distributions of naturally produced fish measured in the tributaries (shaded bars) and in the mainstem trap (solid bars) compared with the acclimated new hatchery stock fish measured in the mainstem trap (open bars). (*b*) Weight distributions of naturally produced fish measured in the tributaries (shaded bars) and in the mainstem trap (solid bars) compared with the acclimated new hatchery stock fish measured in the mainstem trap (open bars).



fish were more common in the tributary traps (Fig. 4). These results indicate that young presmolting fish were moving for several years among different rearing environments within the Hood Basin as they grew. Very young fish were also seen migrating past the mainstem trap, indicating that some of them were leaving the Hood Basin. However, age-0 smolts were absent, whereas age-1 smolts were rare among naturally produced fish based on the age at saltwater entry mark identified on adult scales. This result indicates that about 25% of the naturally produced fish out-migrated from the Hood Basin as presmolts. The nonsmolting juveniles that were leaving the Hood Basin may have died or they may have reared for another year or two in the Columbia before smolting. Larger, age-2 and age-3 juveniles, probably smolts, dominated the mainstem trap in the spring. However, all ages of naturally produced fish, with highly variable sizes and both smolts and presmolts, were moving within the Hood Basin and out of the Hood Basin at all times that sampling occurred (Fig. 5).

These behavior patterns were absent among the hatchery fish (Figs. 4 and 5). They entered the mainstem trap from a few days to a few weeks after release and were large and relatively uniform sized. They were released at age 1 and less than 1% of them were older than age 1 when they entered salt water, as indicated by the entry mark on the adult scales (Fig. 4). Hatchery fish were not found lingering in the Hood Basin either. They were released between the first of April and the middle of May and over 99% of them were gone from the Hood Basin by the end of June based on sampling that continued through October (Fig. 5). Residual hatchery

Fig. 2. (*a*) Comparison of the length distributions of the acclimated new hatchery stock just before release (solid bars) and when captured at the mainstem trap (shaded bars). The distribution measured in the tributary trap was intermediate. The distribution of the naturally produced fish at the mainstem trap is also shown (open bars). (*b*) Comparison of the length distribution of the acclimated new hatchery stock (solid bars), the old hatchery stock (shaded bars), and the naturally produced fish (open bars), all measured in the mainstem trap.



steelhead, which never out-migrate and have been seen near release sites in other Columbia tributaries (e.g., Jonasson et al. 1995; McMichael et al. 2000), have not been observed in the Hood Basin after June, although their presence cannot be completely ruled out.

Survival

The annual survivals and the data from which they were calculated are presented for naturally produced winter and summer steelhead and for the new and old hatchery stocks (Table 3). Averages, standard deviations, and information about statistical significance for each measurement are also presented (Table 4).

Both the new and the old hatchery fish had high egg-to-smolt survivals while they were in captivity compared with wild fish rearing in the natural environment (Tables 3 and 4). Both hatchery stocks had average egg-to-smolt survivals over 60% compared with less than 2% for the naturally produced winter steelhead and less than 1% for the naturally produced summer steelhead (differences between natural and hatchery fish significant at $P < 0.001$). Both hatchery stocks therefore produced significantly more smolt offspring per parent ($P < 0.001$) than did the naturally spawning parents (Table 4).

The survival superiority was reversed between the natural and hatchery groups for smolt-to-adult survivals, which occurred while all of the fish were in the same environment. Naturally produced smolts had average smolt-to-adult survivals of 5–6%, whereas both hatchery stocks had average survivals over the 5 years of about 1% (differences between

Table 2. Comparisons of freshwater and saltwater ages for naturally produced steelhead, the new hatchery stock, and the old hatchery stock.

Freshwater age (years)	Saltwater age (years)	Natural winter steelhead (%)	Natural summer steelhead (%)	New winter hatchery (%)	Old summer hatchery (%)
1	1	—	0.8	1.5	2.9
	2	0.9	0.1	76.7	83.5
	3	0.8	—	19.8	12.6
	4	—	—	—	0.1
	Repeat	0.1	—	1.2	0.8
2	1	4.9	5.9	—	—
	2	59.5	57.4	0.6	0.1
	3	16.5	12.4	0.1	0.01
	4	—	0.2	—	—
	Repeat	5.2	2.9	—	—
3	1	0.5	1.3	—	—
	2	8.9	16.5	—	—
	3	2.4	1.4	—	—
	Repeat	0.1	0.9	—	—
4	2	0.1	0.1	—	—

Note: All measurements are from scales collected from returning adult fish at Powerdale Dam.

Fig. 3. Time of out-migration, measured as date of capture in the mainstem smolt trap averaged over all years of the study, comparing naturally produced fish (solid line) and the new hatchery stock (gray line).

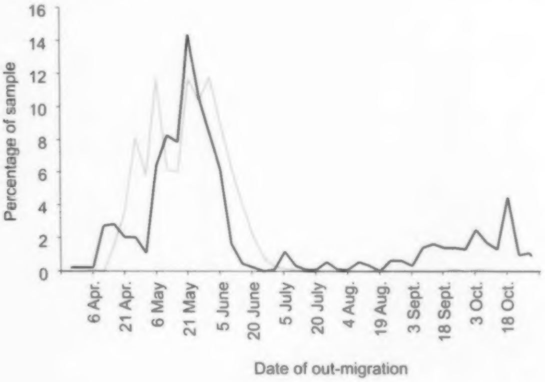
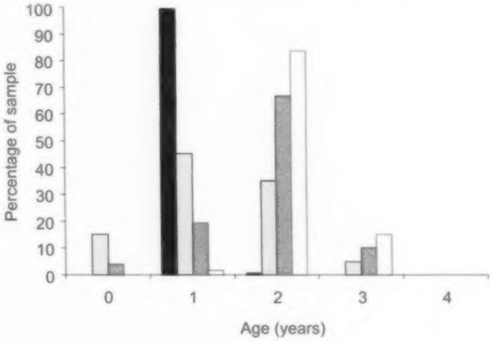


Fig. 4. Ages of juveniles comparing the data for the new hatchery stock measured from adult scales (solid bars) and the naturally produced fish measured in three different samples: tributary smolt traps (light shaded bars), mainstem smolt trap (dark shaded bars), and as adults (open bars). All three naturally produced samples included fish that were age 4 but at less than 1%.

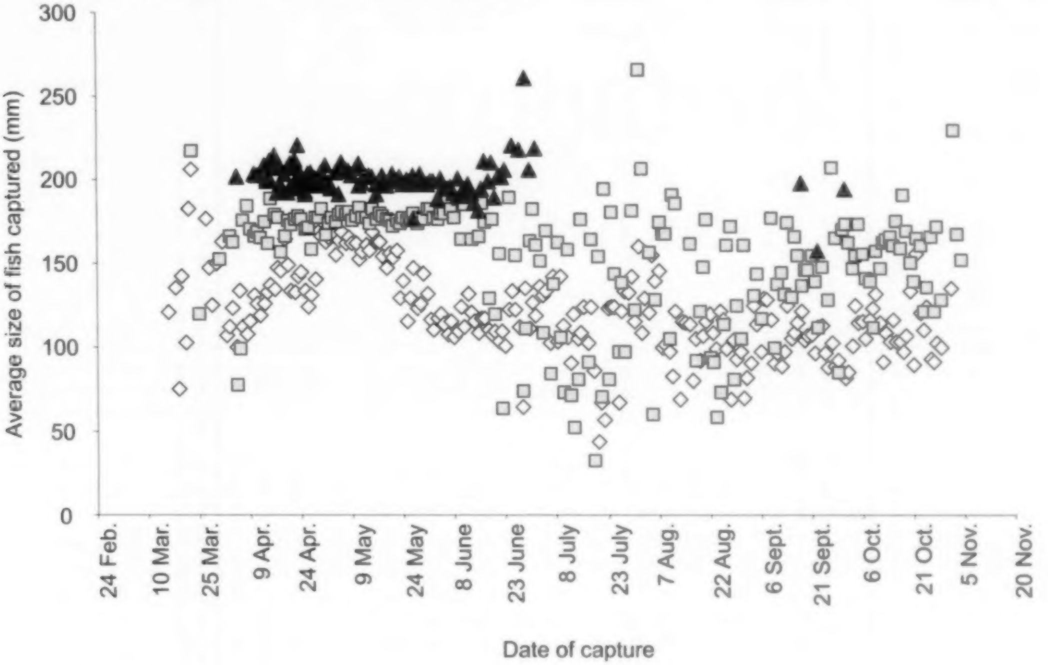


natural and hatchery fish significant at $P < 0.001$; Table 4). However, the survival superiority given the hatchery fish while they were captive continued through to return adults, as they had higher overall egg-to-adult survivals and produced many more adult offspring per parent than the naturally produced fish (both significant at $P < 0.001$; Table 4).

The naturally produced winter steelhead had significantly higher egg-to-smolt survivals, smolt offspring produced per parent, egg-to-adult survivals, and adult offspring produced per parent than the naturally produced summer steelhead (all significant at $P < 0.01$ or greater; Table 4). For example, egg-to-smolt survivals were 10 times higher among the winter steelhead than among the summer steelhead. This difference in survivals was probably related to a difference in the proportion of hatchery parents and the origin of hatchery parents between the two naturally spawning populations. The winter steelhead population included 2–51% hatchery fish, primarily first-generation returns from the local broodstock included among the natural spawners in the last 2 years of the study, whereas the summer steelhead population included 78–88% hatchery fish each year of the study, all from the old hatchery stock (Table 3). The two natural populations had similar smolt-to-adult survivals (differences not significant; Table 4).

The new hatchery stock over the 5 years of the study had significantly fewer smolt and adult offspring per parent and lower smolt-to-adult survivals than the old hatchery stock (significant at $P < 0.05$ or greater; Table 4). For example, over the 5 years, the new hatchery stock produced 35% fewer smolts and 60% fewer adult offspring per parent compared with the old hatchery stock. Comparisons of the direct-released new hatchery stock, the acclimated-released new hatchery stock, and the old hatchery stock demonstrated that the acclimated new hatchery stock had significantly lower smolt-to-adult, egg-to-adult, and adult offspring per parent than either of the other hatchery treatments ($P < 0.05$

Fig. 5. Average length per day by date of out-migration for naturally produced fish in the tributaries (open diamonds) and in the mainstem (shaded squares) and for hatchery fish at the same locations (solid triangles) (both samples combined). Data are averaged over all years of the study. The hatchery fish were over 99% age-1 smolts. The naturally produced juvenile out-migrants ranged from age 0 to age 4 and included both smolts and nonsmolting migrants based on the differences between age at out-migration measured from juvenile scales and age at smolting measured from adult scales.



or greater; Table 4). For example, the average smolt-to-adult survival for the acclimated fish was only about half of what was observed for the direct-released fish and for the old hatchery stock. The direct-released fish and old hatchery stock had similar smolt-to-adult survivals.

Discussion

The new hatchery stock juveniles differed significantly from the naturally produced juveniles in every phenotypic trait that was measured in this study, except saltwater age. The out-migrating new hatchery stock juveniles were larger and less variable in size, younger, and less variable in freshwater and total age, all smolts, whereas the out-migrating wild fish were either smolts or presmolts, out-migrated over a narrow spring window, whereas some wild fish out-migrated throughout the sampling season, and lacked the life history complexity that naturally produced juveniles expressed. The fish from the new hatchery stock more closely resembled the fish from the old hatchery stock, particularly by the time they reached the mainstem trap. The phenotypic differences were probably environmentally caused, since both the hatchery and naturally produced fish were from the same parent gene pool. However, large phenotypic responses by fish from the same parent gene pool to the differences between the captive and natural environments are consistent with the process of domestication (Kohane and Parsons 1989). After the hatchery fish were released, the phenotypic differences provided an opportunity for different responses to selection by the two groups while they were in the same natural environment.

Some of the naturally produced juveniles may have had hatchery parents. Substantial numbers of the old hatchery stock summer steelhead were passed into natural spawning grounds each year and some first-generation winter steelhead adults from the new hatchery stock contributed to the 1997 and 1998 out-migrant years. It was not possible to determine whether the phenotypes of the offspring of naturally spawning hatchery fish differed from those of the offspring of wild fish, since the juveniles caught in the traps could not be assigned to either hatchery or wild parents. It is likely, however, that there were very few offspring of the naturally spawning old hatchery stock in the traps. Although the naturally spawning summer steelhead had very large proportions of old hatchery stock fish among them, their egg-to-smolt survivals were extremely low, only about 10% of what was observed for the naturally spawning winter steelhead. This result indicates reproductive failure by the old hatchery summer steelhead stock, which either did not deposit the eggs that were expected or did not produce juveniles that survived to be captured in the smolt traps. Once summer steelhead smolts reached the mainstem trap, their smolt-to-adult survival was not significantly different from that of the winter steelhead smolts, and the number of wild adult summer steelhead returning annually to the river was fairly constant since the early 1990s. Evidence of reproductive failure by the old hatchery stock in the Hood River is consistent with earlier studies of this same hatchery stock, which demon-

Table 3. Five years of survival data for naturally spawning winter and summer steelhead, the new hatchery stock, and the old hatchery stock in the Hood River.

Parent group	Smolt out-migration year	Number of parents	Hatchery fish among parents (%)	Number of eggs	Number of smolts	Egg-to-smolt survival (%)	Smolt offspring per parent	Number of adult offspring to river mouth	Smolt-to-adult survival (%)	Egg-to-adult survival (%)	Adult offspring per parent to river mouth
Naturally spawning winter steelhead	1994	355	3	751 450	5036	0.67	14.2	282	5.60	0.038	0.79
	1995	306	2	711 550	4672	0.66	15.3	263	5.63	0.037	0.86
	1996	166	3	279 300	7662	2.74	46.2	355	4.63	0.127	2.14
	1997	372	43	665 000	22 550	3.39	60.6	1212	5.37	0.182	3.26
	1998	491	51	1 000 800	14 024	1.40	28.6	1274	9.08	0.127	2.59
Naturally spawning summer steelhead	1994	2211	78	4 857 500	1347	0.03	0.6	156	11.60	0.003	0.07
	1995	1348	82	2 866 500	2883	0.10	2.1	144	4.99	0.004	0.11
	1996	1853	88	3 452 500	5661	0.16	3.1	112	1.98	0.003	0.06
	1997	652	80	1 238 000	3899	0.31	6.0	171	4.39	0.009	0.26
	1998	1493	88	2 936 000	8670	0.30	5.8	212	2.45	0.005	0.14
New winter steelhead hatchery stock	1994	75	0	62 200	38 034	61.20	507.1	354	0.93	0.569	4.72
	1995	43	2	95 000	42 898	45.14	997.6	579	1.35	0.610	13.48
	1996	82	28	63 800	50 904	79.80	620.8	381	0.75	0.598	4.65
	1997	68	46	85 500	59 837	70.01	880.0	323	0.54	0.378	4.75
	1998	58	48	102 500	62 136	60.64	1071.3	445	0.72	0.434	7.66
Old summer steelhead hatchery stock	1994	92	100	161 324	90 042	55.81	976.7	693	0.77	0.430	7.52
	1995	71	100	124 216	76 330	61.45	1075.4	1242	1.63	1.000	17.50
	1996	54	100	94 448	68 378	72.40	1267.0	622	0.91	0.658	11.52
	1997	54	100	94 624	60 993	64.46	1128.0	584	0.96	0.617	10.80
	1998	54	100	93 826	64 910	69.18	1210.7	588	0.91	0.627	10.97

Note: The naturally spawning fish were in the natural environment throughout their life cycle. The hatchery stocks were in a hatchery environment from eggs to smolts and then in the same natural environment as the naturally produced fish from smolts to adults. The proportions of previous-generation hatchery adults that were among the parents of each production year are also included. All data were corrected for age structure and harvest in the lower Hood River.

Table 4. Comparison of the average survivals for naturally spawning winter and summer steelhead, the new hatchery stock, and the old hatchery stock in the Hood River produced from the data in Table 3.

Group	Egg-to-smolt survival, average (SD) (%)	Smolt offspring leaving the river per parent, average (SD)	Smolt-to-adult survival, average (SD) (%)	Egg-to-adult survival, average (SD) (%)	Adult offspring to the river per parent, average (SD)
Naturally spawning winter steelhead	1.77 (1.24)	33.0 (20.1)	6.06a (1.74)	0.102 (0.063)	1.93 (1.08)
Naturally spawning summer steelhead	0.18 (0.12)	3.5 (2.3)	5.08a (3.86)	0.005 (0.003)	0.13 (0.08)
New winter steelhead hatchery (all years combined)	63.36b (12.83)	815.4 (243.8)	0.86 (0.31)	0.518d (0.105)	7.05 (3.81)
New winter steelhead hatchery (direct release)	53.17b (11.36)	752.4 (346.8)	1.14c (0.30)	0.590d (0.029)	9.10e (6.19)
New winter steelhead hatchery (acclimated release)	70.15b (7.82)	857.3 (226.1)	0.67 (0.11)	0.470 (0.114)	5.69 (1.71)
Old summer steelhead hatchery	64.66b (6.50)	1131.6 (113.7)	1.03c (0.34)	0.666d (0.207)	11.66e (3.62)

Note: All pairwise differences within each statistic are significantly different from each other at $P < 0.05$ or greater except the following, which were not significant: natural winter versus natural summer smolt-to-adult (indicated by "a"); all new hatchery treatments versus old hatchery egg-to-smolt (indicated by "b"); new hatchery direct release versus old hatchery smolt-to-adult (indicated by "c"); new hatchery combined years and direct release versus old hatchery egg-to-adult (indicated by "d"); and new hatchery direct release versus old hatchery adult offspring per parent to the river (indicated by "e").

strated that it had low reproductive success in other basins (Chilcote et al. 1986; Leider et al. 1990; Kostow et al. 2003).

Differences in survival measurements for the naturally produced and new hatchery stock juveniles were also significant over all juvenile life stages, indicating that selection was different for the two groups. The naturally produced fish had relatively low egg-to-smolt survivals compared with the high survivals that the hatchery fish experienced while they were in captivity. This difference reflects a relaxation of natural selection on the hatchery fish while the fish were captive so that more fish reached smolting than would naturally do so (Lynch and O'Hely 2001). However, over the 5 years, the elevated egg-to-smolt survivals of the hatchery fish in captivity ranged from only 45% to 80%, which was lower than the 90–95% that is often reported for hatchery programs (ODFW and USFWS 1996). Survival also differed between the groups after the new hatchery fish were released into Hood River, with the advantage going to the naturally produced fish. Average smolt-to-adult survivals for the naturally produced winter and summer steelhead were five to six times higher than for the new hatchery stock.

Based on these two measures of survival, there were two opportunities for modified selection to affect the new hatchery stock. The first occurred while the hatchery fish were under artificial conditions, where selection was relaxed compared with the naturally produced fish but also 20–55% of the hatchery fish were lost each year. The second opportunity occurred after the fish were released when the new hatchery stock had very low smolt-to-adult survivals relative to the naturally produced fish while the two groups were in the same environment.

A third opportunity for modified selection is indicated by the change in length and weight of the new hatchery stock fish from their release to their recapture in the mainstem trap. The smolt traps readily captured very small, young wild fish throughout the sampling season, so it is unlikely that the small hatchery fish were simply not caught in the traps. It is not known whether the smaller hatchery fish grew or died between sample sites. However, substantial growth between sample sites seems unlikely, since the time between release and recapture was only a few days to a few weeks. The old hatchery stock fish took a similar time to reach the mainstem trap but showed no evidence of growth. Further, over 99% of the hatchery fish that returned as adults had out-migrated and entered saltwater as yearling smolts rather than lingering somewhere to grow to an older age. On one hand, the loss of the small fish may be expected. Bigger body size in juvenile fish is generally thought to be advantageous when fish are placed in competition (Abbott and Dill 1989; McMichael et al. 1999). Most studies of steelhead hatchery fish performance have demonstrated that small hatchery steelhead, including any that do not smolt as yearlings, have very poor survival to adults (Wagner et al. 1963; Wagner 1967). Also, the size distributions of the new hatchery stock fish became progressively more similar to those of the old hatchery stock fish as they progressed from release to the mainstem trap. Finally, the treatment that produced the smallest hatchery fish was the acclimated releases of the new hatchery stock and it had the lowest smolt-to-adult survivals observed in this study. The earlier studies, along with the results presented here, suggest that the smaller hatchery fish had a survival disadvantage.

However, although the smallest hatchery fish were not observed after release, the naturally produced out-migrating juveniles were even smaller, yet they were readily captured in the traps throughout the monitoring season and had far higher survival to adults than either hatchery stock. Size of hatchery steelhead may be less important as a direct indication of survival potential than it is an easy to measure consequence of more complicated behaviors and physiological traits. Berejikian et al. (1996) proposed that larger size was a result of greater aggressiveness, which was advantageous in captivity but disadvantageous in the natural environment (also see Huntingford et al. 1990). Perhaps the smaller hatchery fish were lost soon after release because they were poorer competitors during captivity and therefore were in relatively poor condition at release. Larger size, or more specifically rapid growth rate, is associated with the physiological changes that accompany smolting (Mahnken et al. 1982; Dickhoff et al. 1995). It is not natural for all Hood River steelhead to smolt as yearlings, so it was likely that some individuals responded poorly to the manipulations that accelerated smolting.

Modified selection pressures and demographic characteristics of a population may work together to contribute to genetic change in a captive population (Lynch and O'Hely 2001). The characteristics of a hatchery program may cause one mechanism or the other to be more important (Woodworth et al. 2002). Characteristics of the new hatchery stock in the Hood River may have increased the importance of small population effects. The census sizes of both the broodstock and the wild winter steelhead population were small during the 1990s. But also the modified age distribution of the hatchery fish decreased the length of their average generation by 1 year compared with the natural population, which should decrease their effective population size for comparable annual census sizes (Waples 1990). Other studies are currently underway in the Hood River to measure the effective sizes of these population components.

Comparisons of fish from the various hatchery treatments also revealed some differences between the two hatchery stocks and between the different release protocols. The new hatchery stock had very similar phenotypes and survivals when compared with the old hatchery stock, particularly when it was released directly into the Hood River without acclimation. The protocol of acclimating the new hatchery stock in a pond provided with some "enriched environmental features" (Flagg and Nash 1999) was associated with smaller juveniles and with juveniles that had smolt-to-adult survivals that were about half of those observed in the old hatchery stock and in the new hatchery stock when it was released directly. This result is contrary to some expectations (Maynard et al. 1995). The acclimated new hatchery stock juveniles were closer to the average size of the naturally produced fish when they were released, although they were still significantly much larger. However, the changes in their size distributions that occurred from release to recapture in the mainstem trap made them more closely resemble the old hatchery stock by the time they out-migrated, and their smolt-to-adult survivals also deviated further from those of the natural fish.

It appears that the most successful fish in the new hatchery stock generally resembled the old hatchery stock, following the homogenous life history strategy of being large yearling smolts that promptly entered the ocean during a narrow window after release. The complex strategies of wild steelhead juveniles to move from natal areas through a variety of rearing environments over 2 or 3 years with wide individual variation were indicated by our data and are also known to occur in other basins (e.g., Everest 1973) but were not present among the hatchery juveniles. It would be possible to artificially produce some of the variation, for example by releasing 2-year-old hatchery smolts, or smaller smolts, or a mix of smolts and presmolts. But as demonstrated by the apparent consequences for the smaller acclimated hatchery fish released in this study, hatchery fish may be made to "look more like" wild fish without behaving like them or surviving like them.

Further, it is not desirable to remove all survival differences between the hatchery and wild fish. The survival advantage that occurs in the hatchery is necessary for a successful hatchery program regardless of potential consequences. In order for a hatchery program to have any value, it has to be able to produce more adult fish from the parents taken into captivity than would occur in the wild. This requires that egg-to-adult survivals and per capita offspring production be greater for hatchery fish than for wild fish. In spite of some shortcomings, the new hatchery stock evaluated in this study was successful by egg-to-adult survival and per capita offspring measures, as both measures were much higher for new hatchery stock fish compared with naturally produced fish.

In conclusion, this study demonstrated large average phenotype and survival differences between hatchery-produced and naturally produced fish from the same parent gene pool. These results indicate that a different selection regime was affecting each of the groups. The processes indicated by these results can be expected to lead to eventual genetic divergence between the new hatchery stock and its wild source population, thus limiting the usefulness of the stock for conservation purposes to only the first few generations.

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Consequences of potential density-dependent mechanisms on recovery of ocean-type chinook salmon (*Oncorhynchus tshawytscha*)

Correigh M. Greene and Timothy J. Beechie

Abstract: Restoring salmon populations depends on our ability to predict the consequences of improving aquatic habitats used by salmon. Using a Leslie matrix model for chinook salmon (*Oncorhynchus tshawytscha*) that specifies transitions among spawning nests (redds), streams, tidal deltas, nearshore habitats, and the ocean, we compared the relative importance of different habitats under three density-dependent scenarios: juvenile density independence, density-dependent mortality within streams, delta, and nearshore, and density-dependent migration among streams, delta, and nearshore. Each scenario assumed density dependence during spawning. We examined how these scenarios influenced priorities for habitat restoration using a set of hypothetical watersheds whose habitat areas could be systematically varied, as well as the Duwamish and Skagit rivers. In all watersheds, the three scenarios shared high sensitivity to changes in in nearshore and ocean mortality and produced similar responses to changes in other parameters controlling mortality (i.e., habitat quality). However, the three scenarios exhibited striking variation in population response to changes in habitat area (i.e., capacity). These findings indicate that nearshore habitat relationships may play significant roles for salmon populations and that the relative importance of restoring habitat area will depend on the mechanism of density dependence influencing salmon stocks.

Résumé : La restauration des populations de saumons est conditionnée par la possibilité de prédire les conséquences de l'amélioration des habitats aquatiques utilisés par les saumons. Un modèle comportant une matrice de Leslie qui quantifie les transitions entre les nids de fraye, les cours d'eau, les deltas de marée, les habitats côtiers et l'océan chez le saumon quinnat (*Oncorhynchus tshawytscha*) nous a permis de comparer l'importance relative de différents habitats selon trois scénarios de densité-dépendance : indépendance de la densité chez les jeunes, mortalité dépendante de la densité dans les cours d'eau, le delta et la côte et migration dépendante de la densité entre le cours d'eau, le delta et la côte. Tous les scénarios présupposent une dépendance de la densité durant la fraye. Nous avons examiné comment ces scénarios influencent les priorités de restauration des habitats en utilisant une série de bassins versants hypothétiques dont les surfaces d'habitats pouvaient être modifiées systématiquement, d'une part, et les rivières Duwamish et Skagit, d'autre part. Dans tous les bassins versants, les trois scénarios ont une forte sensibilité aux changements de mortalité sur la côte et dans l'océan et ils génèrent des réponses semblables aux changements des autres variables (i.e., la qualité de l'habitat) qui contrôlent la mortalité. Toutefois, les trois scénarios génèrent de remarquables variations dans les réactions de la population aux changements de surface (i.e., la capacité) des habitats. Ces résultats indiquent que les interactions au niveau de l'habitat côtier jouent un rôle significatif chez les populations de saumons. De plus, l'importance relative de la restauration des surfaces d'habitat dépendra des mécanismes de densité-dépendance qui influencent les stocks de saumons.

[Traduit par la Rédaction]

Introduction

Habitat degradation and loss have been listed as major causes of population declines in Pacific salmon (*Oncorhynchus* spp.) (Nehlson et al. 1991). However, it has been difficult to compare the relative effect of freshwater habitat degradation with other factors such as changes in marine survival (Coronado and Hilborn 1998) or harvest (Cass and Riddell 1999), in part because anadromous salmonids use multiple habitats during their life cycle. Attempts to address recovery issues like these typically employ life cycle models because such models integrate survival changes at multiple life stages (Crowder et al. 1994; Caswell 2001). Such models often assume that population dynamics are density independent (Kareiva et al. 2000; Wilson 2003) (Fig. 1*a*), especially when applied to threatened species whose populations are small and for which compensatory interactions are presumed to be negligible. However, where habitat has been

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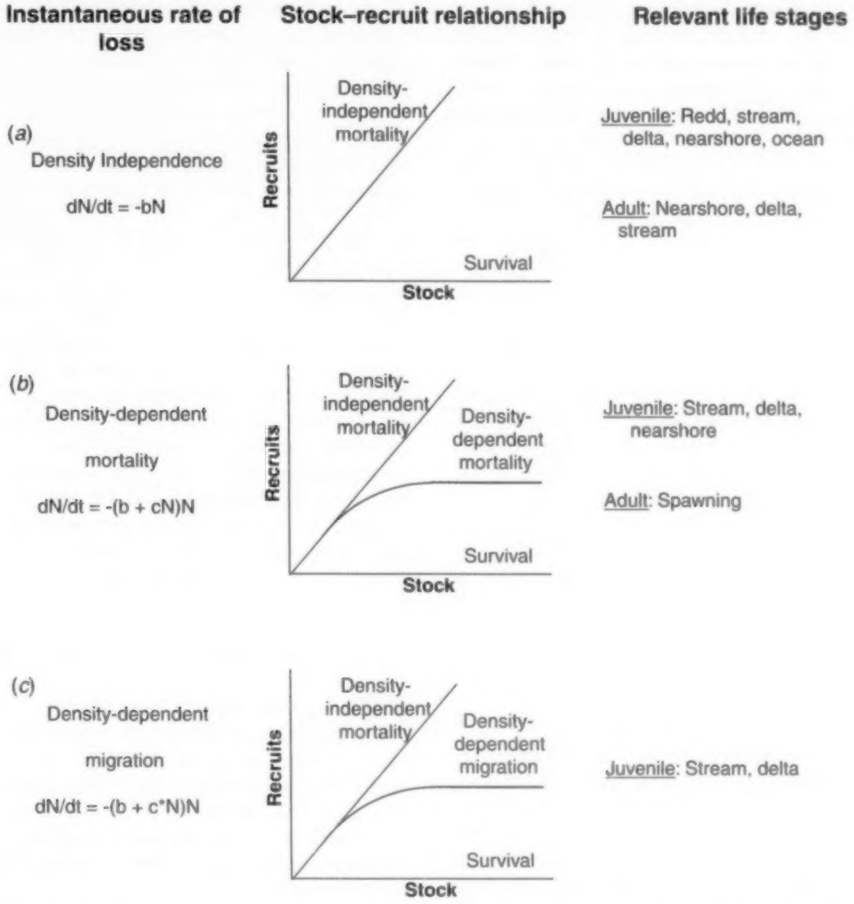
Fig. 1. Three potential scenarios of density dependence, their mathematical formulation, graphical depiction, and relevant possible life stages in this model. In the first two scenarios (density independence and density-dependent mortality), the instantaneous rate of loss refers to instantaneous mortality, where b is the density-independent mortality rate, c is the density-dependent mortality rate, and N is the population size. In the density-dependent migration scenario, the rate of loss is a combination of instantaneous density-independent mortality (b) and density-dependent migration rate (c^*).


Instantaneous rate of loss
Stock–recruit relationship
Relevant life stages
(a)
Density Independence
dN/dt = -bN
Density-independent mortality
Recruits
Survival
Stock
Juvenile: Redd, stream, delta, nearshore, ocean
Adult: Nearshore, delta, stream
(b)
Density-dependent mortality
dN/dt = -(b + cN)N
Density-independent mortality
Density-dependent mortality
Recruits
Survival
Stock
Juvenile: Stream, delta, nearshore
Adult: Spawning
(c)
Density-dependent migration
dN/dt = -(b + c*N)N
Density-independent mortality
Density-dependent migration
Recruits
Survival
Stock
Juvenile: Stream, delta

severely degraded or eliminated, concentration of organisms into remaining suitable habitat may result in compensatory interactions and limit the population at a reduced capacity (Achord et al. 2003). Thus, an incorrect assumption of density independence may erroneously suggest to managers that recovery of habitat area will not benefit populations because improvements can be made only to a population's productivity (habitat quality) and not to capacity (habitat quantity).

Some researchers have integrated density dependence into population viability models to examine how changes in both productivity and capacity affect population response (e.g., Ginzburg et al. 1990; Nickelson and Lawson 1998). Most such models assume that compensation operates directly on survival during a population's residency in a habitat and that instantaneous mortality rate is a second-order function of population size (Fig. 1*b*). This assumption is valid for species that are unable to emigrate from a particular habitat (e.g., Connell 1961) but not necessarily for mobile species such as anadromous salmon for which density dependence may manifest itself in altered migration rates (Reimers 1973; Bradford and Taylor 1997). As they migrate from headwaters to oceans, salmon use multiple habitats, and the existence and form of density dependence may vary depending upon life history and habitat availability. For example, sockeye salmon (*Oncorhynchus nerka*) rear at least 1 year in lakes and, not surprisingly, show some of the most striking patterns of density-dependent mortality (Burgner 1991). In contrast, some stocks of ocean-type chinook salmon (*Oncorhynchus tshawytscha*) appear to respond to increases in density by modifying residency in freshwater and estuarine habitats (Reimers 1973), thereby supporting the existence of density-dependent migration.

Many salmon populations are depressed, and managers are faced with multiple recovery options and the need to understand which habitat restoration actions might yield the greatest population responses. These circumstances motivate the need to carefully examine how different possible mechanisms of density dependence may influence the response of populations to restoration actions. The goals of this paper are to examine the contribution of various aquatic habitats to population dynamics of wild chinook salmon in the Puget Sound region and evaluate how different types of density dependence might change the importance of these habitat-specific effects. We first formulate a modified age-structured Leslie matrix model that specifies redd, stream, tidal delta, nearshore, and ocean transitions. We parameterize the model to represent ocean-type chinook salmon in Puget Sound, which is the dominant life history type in the basin (Healey 1991). With this model, we evaluate how varying assumptions of density dependence alter the estimated importance of different habitats to a population. We examine three possible forms of density dependence: juvenile density independence with a spawning habitat capacity, density-dependent mortality in various juvenile rearing habitats, and density-dependent migration between rearing habitats. We model ocean-type chinook salmon populations from four hypothetical watersheds with differing habitat configurations as well as two real-world examples: the Duwamish and Skagit rivers. For each watershed, we systematically alter survival parameters at each life stage to examine how populations might respond to changes in quality of various habitats. We then systematically vary habitat area for spawning, stream rearing, delta rearing, and nearshore rearing to examine how assumptions of density dependence might alter predictions of population response to habitat restoration actions that increase habitat area. Our purpose is not to recommend specific restoration strategies for particular populations but rather to examine how assumptions of density dependence in different habitats might alter our conclusions about effects of habitat change on a population.

Methods

Puget Sound ocean-type chinook life history

Ocean-type chinook salmon enter Puget Sound rivers between June and September and typically spawn in September and October (Williams et al. 1975; Healey 1991). Eggs develop in the gravel for up to 150 days, remaining in redds even after emergence. After leaving redds, fry migrate downstream over a period of 1–3 months, with most fish reaching the tidal delta (tidally influenced habitats near the river mouth) between February and June. Juveniles reside in the tidal delta for up to 2 months. Juveniles then migrate to nearshore marine habitats such as beaches and eelgrass beds that are subject to freshwater influence, where they remain for approximately 4 months. The vast majority of adults then rear at sea for 3–5 years before returning to spawn, but some individuals spawn at 2 and 6 years of age (Myers et al. 1997; Coronado and Hilborn 1998).

The generalized matrix model

To examine the response of the population to changes in habitat-specific survival rates, we used a modified Leslie matrix model (cf. Sabaton et al. 1998; Kareiva et al. 2000; Gouraud et al. 2001) to track survival of females in the population. This approach specifies age-specific transition probabilities to calculate changes in population size following

$$(1) \qquad \boldsymbol{N}_{t+1} = \mathbf{A}\boldsymbol{N}_t$$

where $\boldsymbol{N}_t$ and $\boldsymbol{N}_{t+1}$ are vectors of population size in year t and $t + 1$ (specific cells in each vector at a particular t are represented as $N_{x,t}$) and $\mathbf{A}$ is a Leslie matrix that incorporates both habitat- and age-specific survival (Table 1).

Leslie matrices usually specify a single survival rate to indicate the transition probability from one age to the next. To incorporate the effects of multiple habitats used within a particular age transition, we subdivided the life cycle into finer intervals based on the residence time (in weeks) in specific habitats. Habitat-specific survival rates are determined by instantaneous (weekly) mortality rates through redds (b_r), streams (b_sj), tidal delta (b_dj), and nearshore habitat (b_nj), where the subscript "j" denotes the juvenile life stage. If residency (in weeks) in these habitats is τ_r, τ_sj, τ_dj, and τ_nj, respectively, survival of juveniles through each habitat is $r = \mathrm{e}^{-b_\mathrm{r}\tau_\mathrm{r}}$ for redds, $s_\mathrm{j} = \mathrm{e}^{-b_\mathrm{sj}\tau_\mathrm{sj}}$ for the stream, $d_\mathrm{j} = \mathrm{e}^{-b_\mathrm{dj}\tau_\mathrm{dj}}$ for the tidal delta, and $n_\mathrm{j} = \mathrm{e}^{-b_\mathrm{nj}\tau_\mathrm{nj}}$ for the nearshore. Total survival for the transition from the first year to the second year is therefore $rs_\mathrm{j}d_\mathrm{j}n_\mathrm{j}$. After the first year, smolts must survive at least 1 year in the ocean (o_x = ocean survival at age x), during which time the population incurs incidental harvest (h, assumed to be constant and not age specific). Each year, a proportion (a_x) of adults mature and return to spawn, with the remainder ($1 - a_x$) staying in the ocean. Spawners must survive terminal harvest (μ, also assumed to be constant and not age specific) and migration through the nearshore (n_a), delta (d_a), and stream (s_a) to reproduce. Like juvenile habitat-specific parameters, survival of adults returning through habitats to spawn is $s_\mathrm{a} = \mathrm{e}^{-b_\mathrm{sa}\tau_\mathrm{sa}}$ for the stream, $d_\mathrm{a} = \mathrm{e}^{-b_\mathrm{da}\tau_\mathrm{da}}$ for the tidal delta, and $n_\mathrm{a} = \mathrm{e}^{-b_\mathrm{na}\tau_\mathrm{na}}$ for the nearshore. These parameters as well as age-specific fecundity (m_x) and the female natal sex ratio (f) determine spawners' contribution to the next generation.

Habitat-specific density dependence

The generalized matrix model assumes density independence at all stages in the life cycle. In addition to a scenario assuming density independence in all juvenile life stages, we examined two additional scenarios: density-dependent mortality and density-dependent migration. All scenarios also assumed density-dependent interactions during adult spawning (see below).

In the density-dependent mortality scenario, the instantaneous mortality rate within a particular habitat increases as a function of density following a Beverton–Holt relationship (Fig. 1b) (also see Moussalli and Hilborn 1986). Beverton and Holt (1957) devised their classic relationship by incorporating residency in particular life stages. The instantaneous density-independent mortality rate b and the density-dependent mortality rate c (per hectare) are used to calculate R, the number of fish surviving a particular life stage. We modified Beverton and Holt's (1957) formulation to separate c from A, the habitat area:

$$(2) \qquad R = \frac{1}{\dfrac{c}{Ab}(\mathrm{e}^{b\tau} - 1) + \dfrac{\mathrm{e}^{b\tau}}{S}}$$

where τ is length of residency in the life stage and S is the initial population size. In this equation, the units of c (hectares per week per fish) are analogous to the amount of area required by an individual per week of residency. A more intuitive and easily measured quantity is the inverse of c (fish

Table 1. Leslie matrix for ocean-type chinook salmon.

Year	1	2	3	4	5	6
1		$o_2a_2n_\mathrm{a}d_\mathrm{a}s_\mathrm{a}fm_2$ $e^{-(\mu+h)}$	$o_3a_3n_\mathrm{a}d_\mathrm{a}s_\mathrm{a}fm_3$ $e^{-(\mu+h)}$	$o_4a_4n_\mathrm{a}d_\mathrm{a}s_\mathrm{a}fm_4$ $e^{-(\mu+h)}$	$o_5a_5n_\mathrm{a}d_\mathrm{a}s_\mathrm{a}fm_5$ $e^{-(\mu+h)}$	$o_6a_6n_\mathrm{a}d_\mathrm{a}s_\mathrm{a}fm_6$ $e^{-(\mu+h)}$
2	$rs_\mathrm{j}d_\mathrm{j}n_\mathrm{j}$					
3		$o_2(1-a_2)e^{-h}$				
4			$o_3(1-a_3)e^{-h}$			
5				$o_4(1-a_4)e^{-h}$		
6					$o_5(1-a_5)e^{-h}$	

Note: The matrix shows transition rates from cohorts in their xth year (columns) to cohorts in their yth year (rows). Total survival in the first year is determined by survival within each habitat (r, redd survival; s_j, juvenile stream survival; d_j, tidal delta survival; n_j, nearshore survival) based on instantaneous (weekly) mortality estimates, as are adult survival rates during return through nearshore (n_a), tidal delta (d_a), and stream (s_a). Mean survival (o_x) and terminal (μ) and nonterminal harvest (h) are based on annual rates. a_x, age-specific breeding propensity; f, female natal sex ratio; m_x, age-specific fecundity.

per week per hectare), the instantaneous capacity per unit of habitat. Therefore, if we define $C = 1/c$ and rearrange terms, eq. 2 can be rewritten as

$$(2a)\qquad R = \frac{SAbC}{S(e^{b\tau}-1) + AbC\,e^{b\tau}}$$

Equation 2*a* can readily be applied to any of the habitats specified in the generalized matrix model by transforming eq. 2 into a survival rate (*R*/*S*). For example, if we assumed that density-independent mortality occurred in redds, then $S = rN_{1,t}$ and

$$(3)\qquad s_\mathrm{j} = \frac{rN_{1,t}A_\mathrm{s}b_\mathrm{s}C_\mathrm{s}}{rN_{1,t}(e^{b_\mathrm{s}\tau_\mathrm{s}}-1) + A_\mathrm{s}b_\mathrm{s}C_\mathrm{s}e^{b_\mathrm{s}\tau_\mathrm{s}}}\Bigg/ rN_{1,t}$$

$$= \frac{A_\mathrm{s}b_\mathrm{s}C_\mathrm{s}}{rN_{1,t}(e^{b_\mathrm{s}\tau_\mathrm{s}}-1) + A_\mathrm{s}b_\mathrm{s}C_\mathrm{s}e^{b_\mathrm{s}\tau_\mathrm{s}}}$$

The equations for each life stage are summarized in Appendix A.

Equation 2 can also be used to model density-dependent migration by assuming that c specifies the rate of individuals migrating from the habitat prematurely (Fig. 1*c*). In this scenario, the number of surviving recruits can be classified into those individuals that remain in the habitat (R_h) and early migrants (R_m). Hence, for any transition through a particular habitat:

$$(4a)\qquad R_\mathrm{h} = \frac{SAbC}{S(e^{b\tau}-1) + AbC\,e^{b\tau}}$$

and

$$(4b)\qquad R_\mathrm{m} = S\sigma - R_\mathrm{h}$$

where σ is the total survival in that habitat (in our analysis, s_j or d_j). The proportion p_h of fish that remain in the habitat for an extended residency is therefore

$$(5)\qquad p_\mathrm{h} = \frac{R_\mathrm{h}}{R_\mathrm{h}+R_\mathrm{m}} = \frac{R_\mathrm{h}}{S\sigma}$$

and the proportion p_m of early migrants is $\sigma - p_\mathrm{h}$ (Fig. 1*c*). The equations for each life stage are summarized in Appendix A.

Applying eq. 5 to the generalized matrix model requires assumptions about the residency time of fish that prematurely move downstream and about the expected survival of different subgroups of migrants. We modeled the residency patterns of these subgroups based on known variation in ocean-type life history. Subyearling juvenile chinook salmon exhibit variation in the extent to which they use stream, delta, and nearshore habitats (Reimers 1973). Reimers (1973) posited the existence of four different subyearling variants utilizing habitats in the Sixes River, and other researchers (e.g., Healey 1991) have observed subsets of these combinations in other watersheds. In the Puget Sound region, three life history subtypes have been observed. These include (*i*) parr migrants, which rear in streams for 3 months before migrating to the nearshore, (*ii*) delta users, which migrate downstream for 1 month, reside in the delta for 1–2 months, and then migrate to the nearshore, and (*iii*) fry migrants, which migrate downstream within 1 month, bypass the tidal delta, and rear in the nearshore (E. Beamer, Skagit System Cooperative, P.O. Box 268, La Connor, WA 98257, U.S.A., unpublished data).

For the purposes of our simulation, we assumed that these three behavioral variants are the result of two stages of migration among habitats following eq. 5. The first stage assumes density-dependent migration in the stream, and the second stage assumes density-dependent migration in the delta. A proportion of fish (p_s, parr migrants) rear in the stream, while the remaining proportion ($s_\mathrm{j} - p_\mathrm{s}$) migrates downstream. Of those migrants, a proportion of fish (p_d, delta users) rear in the delta, while the remaining fish ($d_\mathrm{j} - p_\mathrm{d}$, fry migrants) migrate directly to the nearshore.

Because residency in different habitats differs among the life history variants, survival of certain variants must be adjusted for residency. Delta users are the typical life history type, and their transition probability through life history stages is $rs_\mathrm{j}(s_\mathrm{j} - p_\mathrm{s})p_\mathrm{d}n_\mathrm{j}$. Survival for parr migrants, which we assume spend an additional 4 weeks in the stream before migrating, is reduced by e^{-4b_sj}. The transition probability of parr migrants through juvenile life history stages is therefore $rs_\mathrm{j}p_\mathrm{s}n_\mathrm{j}e^{-4b_\mathrm{sj}}$. Fry migrants spend 4 weeks less in the stream and in the delta compared with delta users and rear instead in the nearshore for 8 weeks. An apparent consequence of reduced stream and delta rearing is higher mortality (following Levings et al. 1989). Based on these assumptions, total survival for fry migrants is $rs_j(s_j - p_s)(d_j - p_d)n_j e^{-8\delta b_{nj} + 4(b_{sj} + b_{dj})}$, where δ is the additional nearshore mortality upon fry migrants. The term $e^{-8\delta b_{nj} + 4(b_{sj} + b_{dj})}$ adjusts survival resulting from both increased mortality of fry migrants in the nearshore and decreased mortality due to reduced residency in streams and delta. We assume the existence of density-dependent mortality during weeks in which all variants reside in the nearshore but density independence during the initial weeks when only fry migrants are present. The combined transition probability from the first to the second year of the entire population is therefore

$$(6)\qquad N_{2,t} = N_{1,t} r s_j n_j [(p_s e^{-4b_{sj}}) + (s_j - p_s)(p_d + (d_j - p_d)e^{-8\delta b_{nj} + 4(b_{sj} + b_{dj})})]$$

Density dependence during spawning

In years during which a large number of spawners return, populations can be limited by the amount of spawning habitat available (Fukushima et al. 1998), which would violate the matrix model's assumption of density independence during adult life stages. We investigated the possibility of density dependence using a Beverton–Holt model that combines the contribution of different age classes to reproduction by assuming that the contribution to fecundity of adult fish that spawn from each age class is proportional to their representation in the total run. The total spawning run in a given year is

$$(7)\qquad T = (1 - \mu) n_a d_a s_a \sum_{x=2}^{6} a_x o_x N_{x,t}$$

The proportion P_x of the total spawning run represented by spawners of a particular age class is therefore

$$(8)\qquad P_x = \frac{a_x o_x N_{x,t}}{T}$$

The Beverton–Holt relationship describing the number of female eggs produced by multiple age-classes is

$$(9)\qquad N_1 = \frac{fT \sum_{x=2}^{6} m_x P_x}{1 + \frac{c_r}{A_r} T}$$

where c_r is the redd capacity (expressed in terms of redds per hectare), A_r is the spawning area (hectares), and $\Sigma_{x=2}^{6} m_x P_x$ is the age-specific fecundity weighted by P_x. Because this weighting is based solely on the representation of an age class in the spawning run, eq. 9 assumes no competitive differences among age classes. We tested an alternate version of the model that included competitive differences, but the results were not qualitatively different from those produced by eq. 9.

Parameterization

We parameterized the model using published estimates of habitat-specific survival, age-specific maturity and fecundity, and other demographic rates (Table 2). Where possible, we used estimates obtained from Puget Sound populations, but other published estimates were used in their absence. Habitat-specific survival parameters were particularly difficult to obtain. We know of no published studies of survival in redds (r) in the Puget Sound region, so we used median estimates of survival obtained from redd-capping studies of chinook salmon on the upper reaches of the Yakima River (Wasserman et al. 1984; Fast et al. 1985, 1986), scaled to the 5-month residency typical of Puget Sound populations. We used the median stream survival (s_j) estimate obtained from a 3-year mark–recapture study of chinook salmon in the Green River that factored in random variability of downstream migration rates (Wetherall 1971). These estimates produced a total freshwater survival of 13.4%, in line with published estimates of freshwater survival (Healey 1991; Bradford 1995) and unpublished estimates of egg–fry survival in the Skagit River during years of moderate incubation floods (C. Greene, unpublished data). Age-specific ocean survival (o_x) was based on current projections of index populations (Chinook Technical Committee 2001), and sensitivity analyses followed baseline projections such that o_3, o_4, o_5, and o_6 were 1.167, 1.33, 1.5, and 1.5 times o_2, respectively. We based terminal (μ) and nonterminal (h) harvest rates on the current projections of 40% and 10% (Chinook Technical Committee 2001). To obtain tidal delta (d_j) and nearshore (n_j) survival, we first calculated estuarine survival by factoring out stream and natural ocean survival of 3-year-olds from published median estimates of survival for coded-wire tagged Puget Sound stocks (Coronado 1995; Coronado and Hilborn 1998) from 1972 to 1988. Estuarine survival was divided into tidal delta and nearshore survival by assuming that (*i*) monthly survival in the tidal delta is 40% greater than nearshore survival (consistent with Healey 1980; Levings et al. 1989) and (*ii*) residency in estuary and nearshore habitats is 1 month greater for wild fish than for hatchery fish (Levings et al. 1986), upon which total marine survival is estimated (Coronado and Hilborn 1998). Total juvenile redd, stream, delta, and nearshore survival were converted to weekly mortality rates using natural log transformations based on published estimates of residency (Table 2). For adult habitat-specific survival, we assumed a 1-week residency in nearshore (Williams et al. 1975) and delta (Simenstad et al. 1982) and a 2-week residency in stream habitats (Williams et al. 1975). Mortality in these habitats was assumed to be low (Ratner et al. 1997) but higher in the nearshore compared with the delta and stream.

Although density dependence can play an important role in the population dynamics of chinook salmon, no habitat-specific estimates of density-dependent parameters exist in the literature to our knowledge. Therefore, we explored the consequences of potential density dependence in different habitats using a heuristic approach. We assumed that capacity increased from stream to delta to nearshore habitat, consistent with differences in individual growth rates in these habitats (Reimers 1973) and life history information indicating that chinook exhibit schooling behaviors during tidal delta and nearshore residence (Reimers 1968; Healey 1991).

Watershed simulations

To better understand how restoration options differ in these watersheds among density-dependent scenarios, we examined a set of simulations in which we systematically varied

Table 2. Model parameters, their estimates, and references for these estimates.

Parameter	Description	Value	Reference(s)
b_r	Weekly redd mortality	0.038	Wasserman et al. 1984; Fast et al. 1985, 1986
b_{sj}	Weekly juvenile stream mortality	0.138	Wetherall 1971
b_{dj}	Weekly juvenile tidal delta mortality	0.113	See text
b_{nj}	Weekly juvenile nearshore mortality	0.185	See text
b_{sa}	Weekly adult stream mortality	0.025	See text
b_{da}	Weekly adult tidal delta mortality	0.02	See text
b_{na}	Weekly adult nearshore mortality	0.1	See text
δ	Fry migrant increase in nearshore mortality	3.333	Levings et al. 1989
τ_r, τ_{sj}, τ_{dj}, τ_{nj}	Juvenile redd, stream, delta, and nearshore residency (weeks)	20, 8, 4, 16	Williams et al. 1975; Alderdice and Velson 1978; Healey 1980
τ_{sa}, τ_{da}, τ_{na}	Adult stream, delta, and nearshore residency (weeks)	2, 1, 1	Williams et al. 1975
o_2, o_3, o_4, o_5, o_6	Annual ocean survival	0.6, 0.7, 0.8, 0.9, 0.9	Chinook Technical Committee 2001
a_2, a_3, a_4, a_5	Age-specific breeding propensity	0.005, 0.097, 0.637, 1.0	Puget Sound TRT, personal communication[a]
m_2, m_3, m_4, m_5, m_6	Age-specific fecundity	2500, 4848, 5710, 6664, 7500	E. Beamer, personal communication[b]
μ	Terminal harvest rate	0.6	Puget Sound TRT, personal communication[a]
h	Nonterminal harvest rate	0.1	Chinook Technical Committee 2001
f	Natal female sex ratio	0.5	
C_r, C_s, C_d, C_n	Redd, stream, delta, and nearshore, capacity	7, 100 000, 200 000, 400 000	
A_r, A_s, A_d, A_n	Redd, stream, delta, and nearshore area (ha)	Table 3	

[a] c/o Mary Ruckelshaus, Northwest Fisheries Science Center, Division of Conservation Biology, 2725 Montlake Boulevard East, Seattle, WA 98112-2097, U.S.A.

[b] Skagit System Cooperative, P.O. Box 268, La Connor, WA 98257, U.S.A.

habitat configuration (amount in each of the four juvenile habitats) such that in any simulation, only one habitat (the "restricted habitat") was a smaller size and all the others were constant at 2000 ha (Table 3). These simulations made no assumptions about which habitat is limiting the population (this an outcome of the model) or the extent to which a particular habitat could be restored.

We also applied our model to two real watersheds, the Skagit and Duwamish rivers. The Skagit River is the largest watershed in Puget Sound and has sustained impacts to some of the headwaters (A_r) as a result of historical logging, mining, and damming, as well as 70–90% loss of rearing habitat in the mainstem (A_s) and delta (A_d) as a result of channelization and diking (Collins et al. 2003). Nearshore habitat area (A_n) may be moderately degraded as a result of shoreline development. However, across the watershed, many of the habitats are still largely intact and functional (Table 3). The Duwamish watershed shares the Skagit's legacy of loss and degradation of headwaters and mainstem habitat but also has undergone recent urbanization resulting in further loss to mainstem rearing habitats, near total loss of the tidal delta, and substantial degradation of nearshore rearing habitats (Table 3) (Simenstad et al. 1982; Collins et al. 2003).

Sensitivity analyses

We evaluated the importance of different habitats on populations by using a simple sensitivity analysis (see Doak et al. 1994). We first established a baseline population trend by simulating dynamics over 300 generations, a more than sufficient amount of time for all runs to equilibrate. We then modified each parameter of interest by 5%, using a decrease in instantaneous mortality for survival parameters and an increase for all other parameters. We then calculated the resulting percent change in the number of spawners resulting from this change. This technique is analogous to the use of elasticity analyses of λ (the dominant eigenvalue of the population matrix) when population dynamics are assumed to be density independent (Doak et al. 1994; Mills et al. 1999; Kareiva et al. 2000).

We evaluated the relative importance of different parameters by comparing population responses with the 5% perturbation. The 5% changes in some demographic parameters could directly correspond to several possible management actions. Increasing redd survival might correspond to better postspawning flow management practices on regulated rivers. Increasing stream survival could correspond to removing rip-rap structures or placing large woody debris as cover, and increasing nearshore survival might result from eelgrass restoration or bulkhead removal. Increases in stream and tidal delta area could result from dam breaching or dike removal, respectively. Changes in other demographic parameters (e.g., ocean survival) make less sense from a management perspective but are useful to gauge ecological or evolutionary responses to environmental changes (e.g.,

Table 3. Habitat areas and the equilibrium spawning population under baseline conditions in six watershed simulations: four model watersheds in which one habitat area is restricted relative to the other three and the Skagit and Duwamish watersheds.

	Model watershed; restricted habitat in					
	Redd	Stream	Delta	Nearshore	Skagit	Duwamish
Parameter						
A_r	500	2 000	2 000	2 000	1 200	375
A_s	2 000	500	2 000	2 000	1 500	570
A_d	2 000	2 000	500	2 000	800	10
A_n	2 000	2 000	2 000	500	8 500	2 000
Baseline equilibrium spawning population						
Juvenile density dependence	349 030	1 396 123	1 396 123	1 396 123	837 674	258 219
Density-dependent mortality	23 620	7 056	19 460	21 799	17 826	1 157
Density-dependent migration	163 519	242 124	171 403	103 494	239 436	10 716

Scavia et al. 2002) relative to changes directly related to management actions.

Results

Baseline population response

In all simulations, the three density-dependent scenarios resulted in markedly different spawning population sizes (Table 3). When density dependence existed solely during spawning, the population increased exponentially until it reached spawning capacity. Not surprisingly, this scenario resulted in the highest equilibrium population size. Under the density-dependent mortality scenario, compensatory processes in stream and delta reduced population growth such that the population size began to level off before it reached spawning capacity. Intriguingly, in the density-dependent migration scenario, the existence of three life history types resulting from density-dependent variation in residency produced between 4 and 34 times the equilibrium population size of the density-dependent mortality scenario, depending upon the habitat configuration (i.e., the distribution of area among habitats). These differences occurred because under the density-dependent migration scenario, the stock–recruit functions specified not mortality but the proportions of the population exhibiting different levels of mortality. This variation enabled a larger total number of fish to exist in different habitats. For example, at high densities, a larger proportion of the population became fry migrants and suffered extremely low survival in the nearshore, but delta residents and stream residents maintained much higher levels. In contrast, in the density-dependent mortality scenario, stock–recruit functions specified mortality on all fish as they migrated through two habitats, resulting in a much lower equilibrium population size.

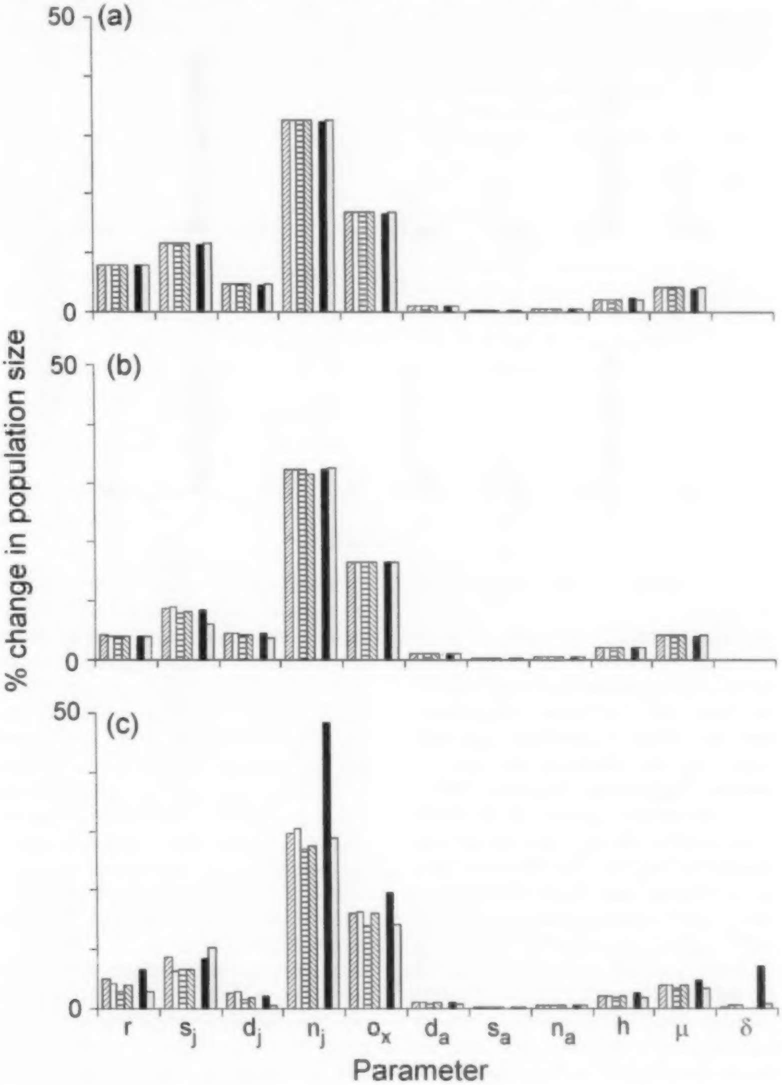
Sensitivity of parameters to perturbation

In the four model watersheds, the contrasting dynamics produced by the three density-dependent scenarios resulted in varied responses to changes in life history parameters. Improvements to survival (Fig. 2) generally had the largest population responses and were qualitatively similar among both scenarios and watersheds. In all scenarios, reducing nearshore (n_j) and ocean mortality (o_x) resulted in the greatest percent change in population size, whereas reducing adult delta (d_a) and stream mortality (s_a) had the smallest effects. Some scenario-specific variation in population sensitivity resulted in slight recovery ranking differences. For example, under the density-dependent mortality scenario (Fig. 2*b*), reducing juvenile delta mortality (d_j) resulted in greater recovery than reducing redd mortality (r), but rankings for these two measures were reversed for the density-dependent migration scenario (Fig. 2*c*).

Quantitative differences in sensitivity to changes in survival among habitat configurations existed primarily for the density-dependent migration scenario (Fig. 2*c*). In habitats subject to the effects of density-dependent migration (stream, delta, and nearshore), sensitivity to changes in habitat-specific survival was lowest when each of those habitats was restricted. This counterintuitive result occurred because density-dependent migration caused fewer individuals to use the restricted habitat, so changes to survival in this habitat benefited a smaller proportion of the population.

The relatively small differences among density-dependent scenarios and model watersheds to changes in survival contrasted with differences resulting from changes in habitat area (Fig. 3). The most obvious difference was the consistently high sensitivity to changes in redd area (A_r) in the juvenile density independence scenario and in stream habitat area in the density-dependent mortality scenario, in contrast with the variable sensitivities across configurations in the density-dependent movement scenario. Under the density-independent scenario, redd area was the only habitat area parameter that could influence changes in population size, so this change mirrored the 5% habitat area increase. Under the density-dependent mortality scenario, changes in population size most strongly depended upon the habitat with the lowest effective capacity (i.e., the capacity after accounting for mortality in previously used habitats). In the case of these four model watersheds, stream habitat always had the lowest effective capacity regardless of which habitat was restricted; spawning capacity was always very large because of the sheer number of adults that could possibly spawn (Table 3, juvenile density independence scenario), and effective capacity in the delta and nearshore remained relatively large even when these habitats were restricted because many juvenile fish died before entering these habitats. As a conse-

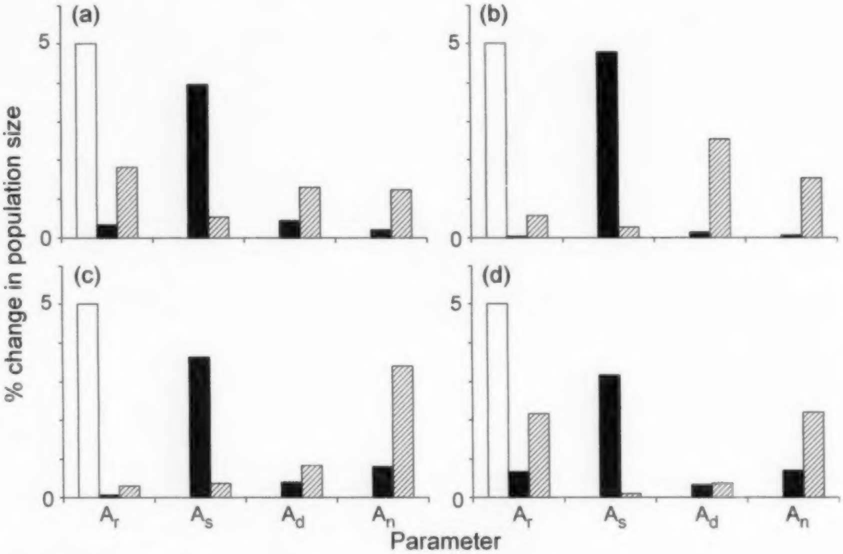
Fig. 2. Sensitivity of population size to changes in survival rates when population dynamics are influenced by juvenile (*a*) density independence, (*b*) density-dependent mortality in the stream, tidal delta, and nearshore, and (*c*) density-dependent migration through the stream and tidal delta. All scenarios incorporate density dependence during spawning. All scenarios are examined in four model watersheds in which redd area (upward-hatched bars), stream area (open bars), delta area (horizontally hatched bars), or nearshore area (downward-hatched bars) is restricted. Also shown are simulations of Skagit (solid bars) and Duwamish (shaded bars). Each bar represents the percent change in the spawning population resulting from a 5% reduction in mortality.



quence, sensitivities to changes in habitat area resulting from the density-dependent mortality scenario showed little variation across habitat configurations. A general conclusion from this finding is that population responses resulting from density-dependent mortality will strongly be influenced by the capacity of single habitats.

Under the density-dependent migration scenario, two habitat configurations caused the restricted habitat to have the highest sensitivity to habitat restoration, while two other configurations caused the habitat "downstream" of the restricted habitat to have the highest sensitivity. Redd and nearshore habitats (Figs. 3*a* and 3*d*) exhibited the first pattern because all fish in the population were affected by those areas (i.e., there was no density-dependent migration out of those habitats). The second pattern was caused by density-dependent migration from stream and delta habitats. When each of these habitats was restricted (Figs. 3*c* and 3*d*), density-dependent migration resulted in shorter residency for portions of the population using this habitat. As a consequence, the downstream habitat had the highest sensitivity to restoration because more individuals were forced to use this habitat. Hence, population responses resulting from density-

Fig. 3. Sensitivity of population size to changes in habitat area when population dynamics are influenced by juvenile density independence (open bars), density-dependent mortality in the stream, tidal delta, and nearshore (solid bars), and density-dependent migration through the stream and tidal delta (hatched bars). All scenarios incorporate density dependence during spawning. All scenarios are examined in four model watersheds in which (*a*) redd area, (*b*) stream area, (*c*) delta area, or (*d*) nearshore area is restricted. Each bar represents the percent change in the spawning population resulting from a 5% increase in a habitat's area. Baseline habitat areas are listed in Table 3.



dependent migration are the outcome of both obligate and variable use of different habitats.

The Skagit and Duwamish simulations revealed similar patterns. As in the simulations of model watersheds, sensitivities to changes in survival were qualitatively similar across density-dependent scenarios and between watersheds (Fig. 2). However, under the density-dependent migration scenario, reductions in delta (d_j) and nearshore mortality (n_j and δ) caused larger relative population increases in the Skagit compared with the Duwamish because of the much higher habitat area available in the Skagit. In contrast with changes in survival, changes to habitat area showed great variation among density-dependent scenarios and between watershed simulations (Fig. 4). In the Skagit River (Fig. 4*a*) under the density-dependent mortality scenario, changes in stream area (A_s) resulted in the greatest sensitivity to habitat change, followed to a lesser extent by changes in delta area (A_d). Changes in other habitat areas had minimal effects. In contrast, under the density-dependent migration scenario, the population showed the greatest sensitivity to changes in delta area, followed closely by redd area (A_d). Changes to stream and nearshore area (A_n) had smaller but not insignificant effects. In the Duwamish River (Fig. 4*b*), density-dependent mortality resulted in a much greater sensitivity to changes in delta area and to a lesser extent to changes in stream area. This pattern was reversed for the density-dependent migration scenario. Both scenarios shared minor sensitivities to changes in redd and nearshore habitat area.

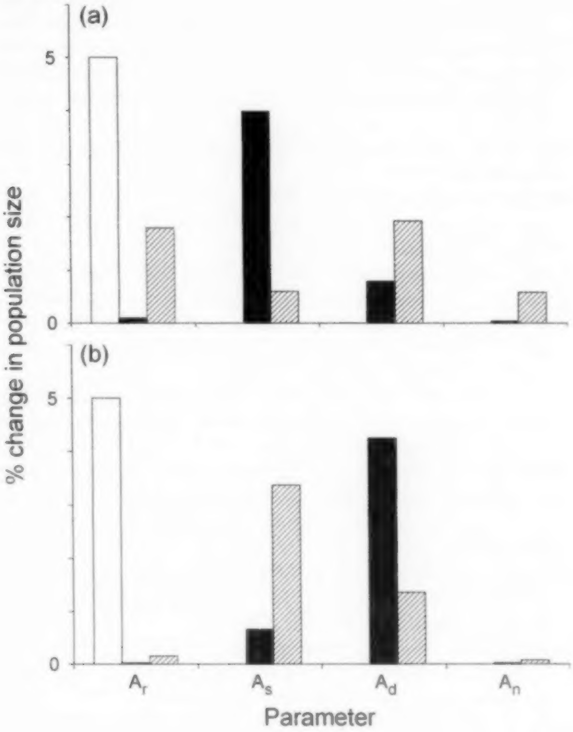
Discussion

Using a population model that disaggregates the chinook life cycle into relevant stages of habitat use, we found that understanding how restoration actions may affect salmon populations depends upon knowledge of density-dependent mechanisms. Assuming density-dependent mortality at all life stages necessarily implies a constant loss of individuals at all life stages (Moussalli and Hilborn 1986). However, if other mechanisms such as density-dependent migration exist, the population may not only equilibrate at a larger size but also respond differently to restoration of habitat.

That different density-dependent mechanisms produce different population responses has long been known in fisheries biology (Ricker 1954; Beverton and Holt 1957). However, its application to population viability analysis and restoration ecology has received much less attention, in part because of the focus on threatened populations, which are often assumed to experience less density dependence. This assumption may actually not be the case if habitat has been lost or degraded, which could result in a resetting of carrying capacity at a lower level (Achord et al. 2003). In such circumstances, alternative density-dependent mechanisms deserve examination to accurately predict population response to restoration.

Our simulations of model watersheds reveal several important generalizations about population responses to restoration. First, the concepts of "habitat limitation" and "limiting factors" are an outcome of both habitat availability and response of the population to habitat availability. For example, density-dependent mortality resulted in a significant population response only to stream habitat restoration, regardless of which habitats might appear in our eyes to be "limited". In contrast, density-dependent migration resulted in increased sensitivity to restoration of habitats downstream of those that we might consider as "limiting" the population. Second, populations may respond very differently to changes in sur-

Fig. 4. Sensitivity of population size to changes in habitat area in the (*a*) Skagit and (*b*) Duwamish rivers when population dynamics are influenced by juvenile density independence (open bars), density-dependent mortality in the stream, tidal delta, and nearshore (solid bars), and density-dependent migration through the stream and tidal delta (hatched bars). All scenarios incorporate density dependence during spawning. Each bar represents the percent change in the spawning population resulting from a 5% increase in a habitat's area. Baseline habitat areas are listed in Table 3.



vival (habitat quality) and habitat area (capacity). For example, all scenarios predicted the highest sensitivity to changes in nearshore survival but often much lower sensitivity to changes in nearshore habitat area relative to that of other habitats. Consequently, recovery plans need to carefully consider how restoration influences demographic parameters. Third, because the distribution of habitats can influence sensitivity of the population to changes in habitat area, recovery options in one watershed may have limited application to other watersheds, even if survival rates in the watersheds are similar.

These issues are highlighted in our real-world simulations of the Skagit and Duwamish watersheds. In both watersheds, delta habitat has undergone much more loss than other habitats. However, the distribution of watershed area differs among spawning, stream, delta, and nearshore habitats. These differences create contrasting responses to changes in habitat area and offer completely opposite predictions of restoration potential under both density-dependent mortality and migration scenarios. These examples point to the critical need for not only empirical assessments of habitat-specific survival rates but also studies of density dependence in systems of interest.

Our results also indicate that some parameters appear to have particular importance for population dynamics, regardless of the existence and mechanism of density dependence. All model scenarios indicated that habitats used by juveniles were the most important for population dynamics, consistent with other population models of salmon (e.g., Nickelson and Lawson 1998; Kareiva et al. 2000). More specifically, we found that population size was most sensitive to changes in nearshore and ocean survival in all model scenarios. These findings agree with empirical studies of large population responses to changes in marine conditions (Beamish et al. 1995; Coronado and Hilborn 1998; Hollowed et al. 2001). Empirical studies of nearshore survival and residency are notably lacking, but the little that has been documented indicates that the nearshore period is associated with large increases in body size (Levings 1994; Korman et al. 1997) and high risk of predation (Healey 1980; Bravender et al. 1999). The fact that the largest increase in population size resulted from a change in nearshore survival suggests that nearshore conditions may be an important limiting factor in the life cycle of ocean-type chinook salmon.

The finding that survival rates largely do not qualitatively vary among density-dependent scenarios is good news for many population viability analyses that have focused on assessing the consequences of variation in survival and have ignored density dependence (Crowder et al. 1994; Doak et al. 1994; Kareiva et al. 2000). Our results suggest that if the alternative recovery actions being examined target primarily survival, conclusions about the efficacy of particular actions may be robust to the particular mechanism of density dependence. However, as many restoration projects seek to increase the amount of high-quality habitat, population viability assessments of alternative habitat restoration projects need to think carefully about changes in density in response to increases in habitat availability. For example, the ongoing debate on the merits of dam removal for listed stocks in the Columbia River (e.g., Kareiva et al. 2000; Wilson 2003) has largely ignored the huge increases in rearing and spawning habitat capacity that will accompany increases in survival if dams are removed. Listed stocks do exhibit density dependence at various stages of their life cycle (Petrosky et al. 2001; Achord et al. 2003), even at their reduced population size. Hence, the long-term consequences of dam removal for population recovery deserve more careful attention as to how stocks will respond to capacity changes after dam removal.

Our analysis provides one way of examining specific assumptions of density dependence and suggests that regardless of the density-dependent mechanism, improvements to nearshore survival will offer disproportionate benefits to ocean-type chinook salmon populations in Puget Sound. However, the analysis begs the question whether a significant decrease in nearshore mortality is feasible. The extent to which we have modified the conditions of these habitats and how these conditions can be improved is largely unknown. Like other aspects of the marine phase of salmon migration, habitat use and survival in the nearshore remain difficult to quantify. Beaches as well as eelgrass and kelp beds have been cited as important nearshore habitats for chinook and other salmon (Healey 1980; Levings et al. 1991;

Korman et al. 1997), and their modification via habitat loss (e.g., via dredging and dock building), bulkhead placement, and alteration of the terrestrial margin have been suggested as having potentially significant impacts on juvenile salmon (Korman et al. 1997). Our analysis suggests that efforts to characterize nearshore habitats, their effects on salmon, and the extent to which they have been modified will significantly enhance our understanding of the population dynamics of chinook and other salmon.

Similar arguments can be raised about ocean survival, the second most important parameter in all model scenarios. Here, our knowledge is even murkier, focusing almost solely on climatic effects on survival (e.g., Kope and Botsford 1990; Mueter et al. 2002). As the scales at which we measure changes in ocean mortality can be on the order of decades and of thousands of kilometres, and its variability can be large (Hobday and Boehlert 2001), ocean survival is largely viewed as out of our direct control. If this is true, ocean mortality must be seen as a constraint reducing the realm of possible management actions to more local habitats. Note, however, that if much of the marine mortality occurs in nearshore habitats (as our results suggest), creative habitat restoration attempts may actually be highly successful.

The most commonly suggested habitat restoration attempts concern improvements to freshwater conditions (e.g., rip-rap removal, placement of large woody debris, and removal of barriers to spawners) and to conditions in the tidal delta (e.g., dike removal and riparian revegetation). Our analysis revealed that the extent to which these changes will improve population size might largely depend upon the mechanism of density dependence and whether these restoration efforts improve habitat quality or quantity. Hence, improved understanding of the existence and mechanisms of density dependence should have important implications for the ways in which we manage habitat for juvenile salmon.

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Appendix A

Table A1 appears on the following page.

Table A1. Equations describing life stage transitions through five life stages in the three scenarios of density dependence.

	Scenarios		
Life stage	Juvenile density independence	Density-dependent mortality	Density-dependent migration
Adult spawning	$N_1 = \dfrac{fT\sum_{x=2}^{6} m_x P_x}{1 + \dfrac{c_r}{A_r} T}$	$N_1 = \dfrac{fT\sum_{x=2}^{6} m_x P_x}{1 + \dfrac{c_r}{A_r} T}$	$N_1 = \dfrac{fT\sum_{x=2}^{6} m_x P_x}{1 + \dfrac{c_r}{A_r} T}$
Incubation	$R_r = rN_{1,t}$ $r = e^{-b_r\tau_r}$	$R_r = rN_{1,t}$ $r = e^{-b_r\tau_r}$	$R_r = rN_{1,t}$ $r = e^{-b_r\tau_r}$
Stream residence	$R_s = s_j R_r$ $s_j = e^{-b_{sj}\tau_{sj}}$	$R_s = \dfrac{R_r A_s b_{sj} C_s}{R_r(e^{b_{sj}\tau_{sj}} - 1) + A_s b_{sj} C_s e^{b_{sj}\tau_{sj}}}$ $s_j = R_s/R_r$	$R_s = \dfrac{R_r A_s b_{sj} C_s}{R_r(e^{b_{sj}\tau_{sj}} - 1) + A_s b_{sj} C_s e^{b_{sj}\tau_{sj}}}$ $s_j = e^{-b_{sj}\tau_{sj}}$ $p_s = R_s/R_r s_j$ $p_m = s_j - p_s$
Delta residence	$R_d = d_j R_s$ $d_j = e^{-b_{dj}\tau_{dj}}$	$R_d = \dfrac{R_s A_d b_{dj} C_d}{R_s(e^{b_{dj}\tau_{dj}} - 1) + A_d b_{dj} C_d e^{b_{dj}\tau_{dj}}}$ $d_j = R_d/R_s$	$R_d = \dfrac{R_s A_d b_{dj} C_d}{R_s(e^{b_{dj}\tau_{dj}} - 1) + A_d b_{dj} C_d e^{b_{dj}\tau_{dj}}}$ $d_j = e^{-b_{dj}\tau_{dj}}$ $p_d = R_d/R_s d_j$ $p_m = d_j - p_d$
Nearshore residence	$R_n = n_j R_d$ $n_j = e^{-b_{nj}\tau_{nj}}$	$R_n = \dfrac{R_d A_n b_{nj} C_n}{R_d(e^{b_{nj}\tau_{nj}} - 1) + A_n b_{nj} C_n e^{b_{nj}\tau_{nj}}}$ $n_j = R_n/R_d$	$R_n = \dfrac{R_d A_n b_{nj} C_n}{R_d(e^{b_{nj}\tau_{nj}} - 1) + A_n b_{nj} C_n e^{b_{nj}\tau_{nj}}}$ $n_j = e^{-b_{nj}\tau_{nj}}$ $p_n = R_n/R_d n_j$ $p_m = n_j - p_n$
Total first-year migration	$N_{2,t} = rs_j d_j n_j N_{1,t}$	$N_{2,t} = rs_j d_j n_j N_{1,t}$	$N_{2,t} = N_{1,t} rs_j n_j[(p_s e^{-4b_{sj}}) + (s_j - p_s)(p_d + (d_j - p_d)e^{-8\delta b_{nj} + 4(b_{sj} + b_{dj})})]$

Shift in wetland plant composition and biomass following low-level episodes in the St. Lawrence River: looking into the future

Christiane Hudon

Abstract: The effects of a 1-m drop in average water levels in 1999 on species composition and biomass were documented for a St. Lawrence River wetland and compared with a similar episode in 1931. These observations highlight the manifold effects of past and future water level fluctuations on St. Lawrence River wetlands and faunal habitats, resulting from natural hydrologic variability, climate change, and (or) human intervention. In 1931 and 1999, waters were 2–3 °C warmer than the previous 10-year average. Low water levels markedly altered wetland vegetation: various Graminea (including *Phalaris arundinacea* and *Phragmites australis*) and facultative annual species invaded previously marshy areas. Submerged species previously found in shallow waters were replaced on dry ground by annual terrestrial plants; *Alisma gramineum* colonized emergent waterlogged mudflats. The low water levels of 1999 induced a spatially discontinuous plant biomass that was richer in terrestrial material than in previous years (1993–1994). In comparison with the 1930s, recent surveys indicate a decline of assemblages dominated by *Equisetum* spp. and *Najas flexilis* and a rise of those dominated by *Lythrum salicaria*, *Potamogeton* spp., and filamentous algae. These shifts reveal the additional effects of nutrient enrichment, alien species, and shoreline alteration accompanying a change from a mostly agricultural to a mostly urbanized and industrialized landscape.

Résumé : Les effets sur la composition spécifique et la biomasse des milieux humides d'une baisse de niveau du Saint-Laurent en 1999 ont été comparés avec un épisode semblable en 1931. Ces observations soulignent l'importance du niveau d'eau sur les milieux humides et les habitats fauniques du Saint-Laurent, résultant de la variabilité hydrologique naturelle, des changements climatiques et (ou) des interventions humaines. En 1931 et en 1999, la température de l'eau était plus élevée de 2–3 °C que la moyenne des 10 années précédentes. Les bas niveaux ont modifié dramatiquement la végétation des milieux humides : diverses graminées (incluant *Phalaris arundinacea* et *Phragmites australis*) et espèces annuelles facultatives ont envahi les anciens marais. Les plantes submergées ont été remplacées par des plantes annuelles terrestres croissant sur les vasières asséchées; *Alisma gramineum* a germé et fleuri sur la vase humide. Les bas niveau de 1999 ont généré un couvert littoral discontinu pour la faune, comportant une biomasse terrestre supérieure à celle de 1993–1994. En comparaison avec les années 30, les données récentes indiquent une diminution des assemblages dominés par *Equisetum* spp. et *Najas flexilis* et un accroissement de ceux dominés par *Lythrum salicaria*, *Potamogeton* spp. et les algues filamenteuses. Ces changements révèlent les effets additionnels de l'eutrophisation, des espèces exotiques et de l'altération des rives accompagnant le passage de la vocation principalement agricole à la vocation urbaine et industrielle des terres avoisinantes.

Introduction

The regulation of large rivers for flood control, commercial navigation, and hydroelectricity production modifies riverine flow patterns by reducing the amplitude and the duration of spring runoff and raising low water levels in late summer (Toner and Keddy 1997). How the alteration of large river discharge is manifested, however, is largely determined by the natural variability of the precipitation to the watershed, which, in the case of the Great Lakes – St. Lawrence River, responds at the continental scale. Flow management in eastern North America is likely to become severely constrained in the future as climate change scenarios come into play; in the North American Midwest, predicted increases in air temperature and evaporation will reduce the overall water supply (Mortsch and Quinn 1996; Natural Resources Canada 2002). Under such conditions, the regional demands for Great Lakes water and the regulation of flow downstream are likely to increase, further altering hydrologic regime and water availability for natural ecosystem components.

Wetlands occupy the shoreline fringe of the transition zone between dry land and purely aquatic systems; as such, the persistence and diversity of this ecosystem is intimately

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linked to water level variations (Bedford et al. 1976; Auble et al. 1994). In temperate latitudes, wetland ecosystems have evolved around predictable seasonal conditions related to the timing, frequency, and amplitude of water level changes, all of which are increasingly threatened by human-induced climate change and management interventions (Mortsch 1998).

Before 1960, the annual range of water levels in the St. Lawrence River near Montreal averaged 6 m (Fig. 1). Since 1960, river discharge has been regulated to optimize hydroelectricity production, control flooding, and maintain water levels for commercial ship traffic. Regulation, variable water supply, and riverbed alteration decreased the annual range of water level variations by half, cutting maximum daily flood levels to about 8.8 m (Fig. 1). Although regulation causes only minor variations in comparison with natural climate-induced interannual variability in discharge, human intervention can either amplify or reduce the effects of extreme high and low levels on wetlands and thus plays an important role in cumulative anthropogenic effects on natural ecosystems.

The effects of various hydrological characteristics on the composition and biomass of riverine wetland species are well documented (Hudon 1997; Toner and Keddy 1997; Jansson et al. 2000); water depth produces known variations in wetland plant morphology (Marie-Victorin 1995), whereas the seasonal range in levels and flows induces shoreline plant zonation (Tessier et al. 1984), influences riparian plant succession (Auble et al. 1994; Shafroth et al. 2002), and determines species richness (Keddy and Reznicek 1986; Nilsson and Keddy 1988). In addition, interactions between water level and combinations of hydrological variables such as water depth/light intensity, litter removal by spring flood, and the fertility/exposure gradient produced by waves and flowing water were shown to control wetland vegetation in the Ottawa (Day et al. 1988) and St. Lawrence rivers (Hudon et al. 2000). Such alterations in species composition, productivity, and distribution of wetland plants are expected to modify markedly the quality of habitats available for aquatic fauna.

Episodes of low annual mean water levels were observed in the 1930s (1931 and 1935), the 1960s (1962–1965), and the late 1990s (1995, 1999, and 2001) (Fig. 1). According to Marie-Victorin (1943), the water levels of 1931 were exceptional, prompting his 1943 study documenting the shift in wetland species composition under low-level conditions in the Montreal area. In addition to the rare opportunity to compare conditions almost 70 years apart, the 1999 low water level episode permitted the testing of four hypotheses regarding the mechanisms by which wetlands respond to a drop in water levels. First, emergent plants should produce more belowground biomass under dry conditions (Tiner 1999). Second, the proportion of emergent, submerged vascular plants and algae found in aboveground biomass should change with low level. Third, the shift in aboveground biomass should reflect taxa's preference or tolerance (as defined by Reed 1988) for the new environmental condition. Fourth, species that invade wetlands under low-level conditions should demonstrate a higher capacity to monopolize light and space than native species. The present study compares climate and water level conditions in 1931 and 1999 and reports on their effects on wetland zonation and biomass, providing insights into the possible outcome of more frequent episodes of low water levels, resulting from climate change, in the Great Lakes – St. Lawrence River basin.

Materials and methods

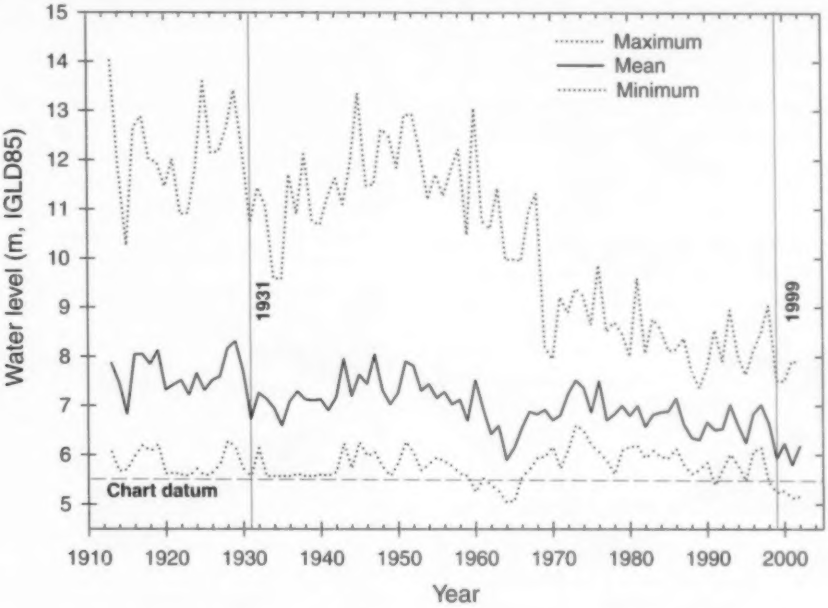
Description of the study area

The study site is located on the St. Lawrence River at the western (upstream) tip of the Tailhandier flats (Boucherville Islands). Located 1 km across from the Port of Montreal, it is the largest wetland (154 ha) in the greater Montreal area. This wetland has been protected from direct human intervention since 1998 through a 20-year lease between the Montreal Port Authority and the Canadian Wildlife Service. This natural habitat, supporting populations of fish, waterfowl, muskrat, beaver, and deer, contrasts sharply with the adjacent, heavily urbanized Montreal area (population 2.5 million). In the 1930s, most of the Montreal area was agricultural; urbanized areas covered only 135 km^2 (27%) of the island of Montreal in 1932, but by 1989, they monopolized 334 km^2 (73%) (Figs. 2*a* and 2*b*).

The Boucherville wetland is characteristic of the low-lying islands of the St. Lawrence Lowlands, a flat landscape of clay deposits intersected with moraine landforms. Bottom sediments generally consist of fine silt and sand, with a locally variable organic component, lying on glacial deposits of marine clay. Emergent marsh vegetation colonizes the shallow (<2 m) shelf around islands and shoals, which drops sharply along a steep slope to deeper waters (Hudon 1997).

Low water level conditions were also observed in 1931 at the Boucherville Islands, along the south shore of Longueuil, and at nearby Île au Vert (Île Plate) (Fig. 2*c*) (Marie-Victorin 1943). This area was significantly altered by major construction projects in the 1960s, including the excavation of the Port of Montreal, the St. Lawrence Seaway, and the Louis-Hippolyte Lafontaine Tunnel. Dredged material was used to shield the nearby shoreline, build up jetties and islands, fill in wetlands, and develop highways and urban areas along the Longueuil–Boucherville shoreline (Figs. 2*c* and 2*d*). Such alterations in the bathymetry and shoreline profile most likely intensified the passage of water in the port area, with a concomitant reduction in flow through the channels between the Boucherville Islands. Sediment buildup in the side channels was also favoured by the reduction of flushing during winter and spring floods (Fig. 1) and the increasing control of ice jams since the beginning of the 20th century. Accordingly, the reduction in water circulation and the progressive drying up of the inner channel increased the surface area of emergent wetlands from about 180 ha in 1931 to about 315 ha in 1996–2000. The 1996 remote sensing image (17 October 1996: 6.374 m) of the Boucherville Islands shows the extensive development of emergent plants in the longitudinal channel (originally called "Chenal du courant" (Channel of the current)) in comparison with the wide-open water channel seen in the 1931 aerial photographs (6 May 1931: 6.704 m) (Figs. 2*c* and 2*d*). Although the 1996 image was taken when the water level was 33 cm lower than when the 1931 image was taken and when the emergent vegetation was well developed (early fall), the proliferation of dense *Typha angustifolia* and of *Phragmites australis* (since 1980, C. Hudon, unpublished data) across the upstream half of the

Fig. 1. Annual mean and minimum and maximum daily water levels recorded in the St. Lawrence River (Port of Montreal, Jetty No. 1) for the period 1913–2002. Vertical lines indicate the years 1931 and 1999; the broken horizontal line indicates the level of reference of navigation charts (chart datum). IGLD85, International Great Lakes Datum of 1985.



channel is an additional indication of its progressive clogging.

The low water levels of 1931 coincided with the well-documented "dust bowl" drought period that reduced water levels in the Great Lakes and threatened agriculture in the Prairies (Rosenzweig and Hillel 1993). In 1999, low water level conditions in the St. Lawrence coincided with a 10% reduction in precipitation and increasing evaporation resulting from a 1–2 °C increase in air temperature in the Great Lakes watershed since 1998. Such changes are on the same order of magnitude as those anticipated in the 2× CO_2 warming climate scenario (Mortsch and Quinn 1996) and could result in more frequent episodes of low levels in the St. Lawrence River (Natural Resources Canada 2002).

Physical and climatological measurements

Changes in land use and shoreline morphology between past (1932) and recent (1989) conditions on the Boucherville Islands were demonstrated using city maps (Divay and Gaudreau 1992) and LANDSAT imagery (obtained from G. Létourneau, St. Lawrence Centre, Environment Canada, 105 McGill St., Montreal, QC H2Y 2E7, Canada, personal communication), respectively. Alteration of the shoreline and land use patterns in the Boucherville Islands were documented from black and white aerial photographs taken on 6 May 1931 (water level 6.704 m) and from multispectral videography taken on 17 October 1996 (water level 6.374 m). Videography is a remote sensing technique using continuous, high-resolution frame acquisition in a video mode.

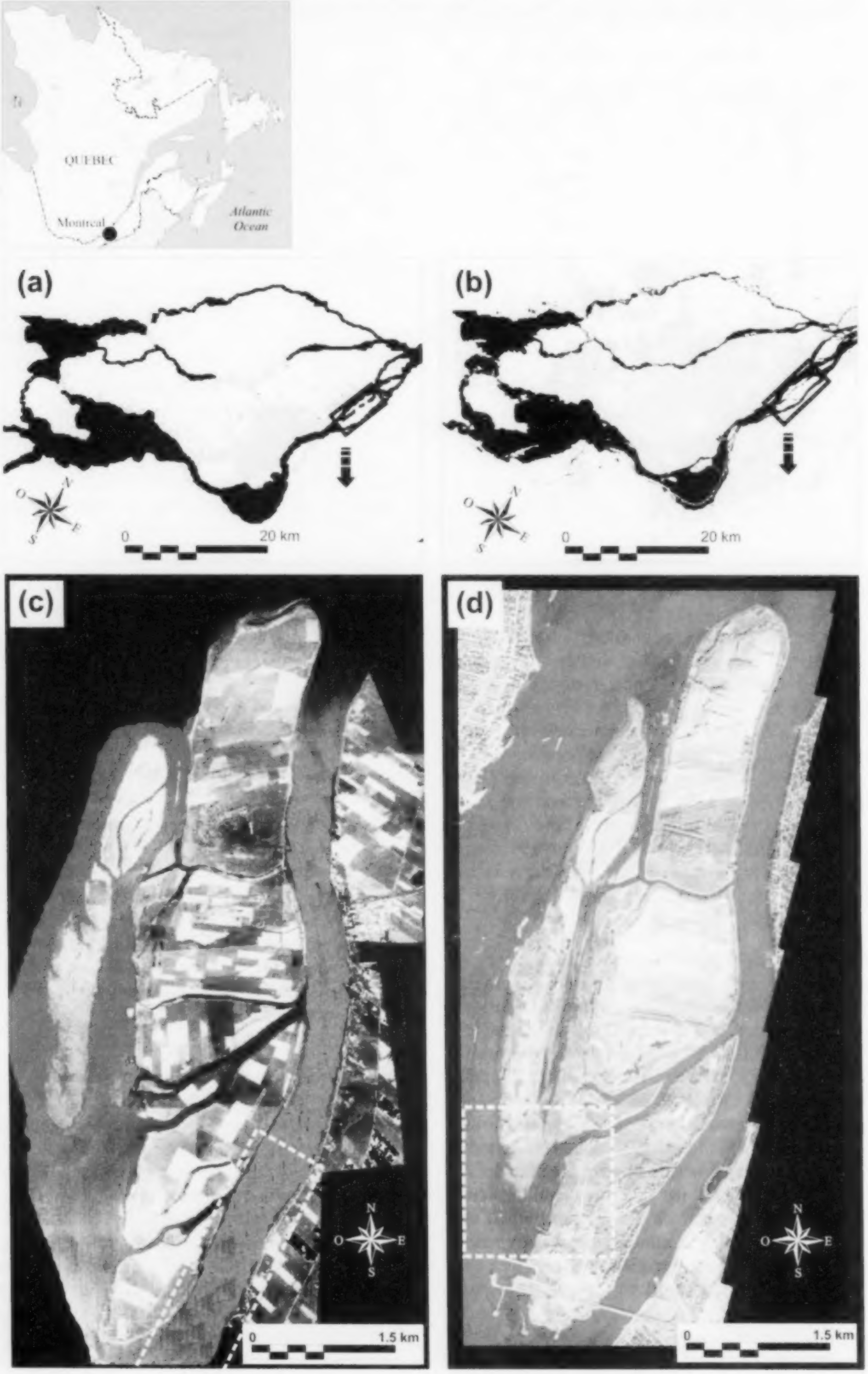
Water level conditions for the 1913–2002 period were obtained from measurements and historical values for the Port of Montreal's Jetty No. 1 (gauging station No. 15520, 5.560 m, referenced to the International Great Lakes Datum of 1985 (IGLD85)), which is 19 cm below the average sea level datum (5.579 m) at that gauging station (Fisheries and Oceans Canada 2002). The elevation of each sample was measured at collection time. Underwater samples were measured using water depth, and samples above the water line were measured with a surveyor's level referenced to the current daily water level. After correction for daily level variations, the elevation of individual samples was reported in metres above the IGLD85 reference level.

Climate and water temperature conditions associated with years of low water levels (1931 and 1999) and the previous 10 years were obtained, respectively, from average daily air temperature and total daily precipitation data recorded at the McGill (1921–1931) and Dorval (1989–1999) weather stations (Environment Canada 2002) and from monthly average water temperature data for the 1921–1999 period obtained from the Atwater water filtration plant (City of Montreal) (Hudon et al. 2003).

Wetland plant composition and biomass

Records of species composition during the low-level years of 1931 and 1999 were compared with each other and with those of previous years. The wetland was divided into five elevation zones (strata) corresponding to different immersion regimes. Under average water level conditions, these elevations coincided with the sequence of wet meadow, marsh, and increasingly deep open water; under low water levels (1931 and 1999), the same elevation sequence coincided with dry meadow, wet meadow, a dry/waterlogged barren mud zone, and shallow open water. In the dry 1999 summer, the two uppermost strata were flooded only in the spring

Fig. 2. Maps of the study area comparing the extent of urbanization on the island of Montreal in (*a*) 1932 and (*b*) 1989. Alteration of the shoreline and land use patterns in the Boucherville Islands are shown for (*c*) 1931 and (*d*) 1996. Enclosed areas on Figs. 2*c* and 2*d* indicate the study sites of Marie-Victorin (1943) and of the present study, respectively.


QUEBEC
Montreal
Atlantic Ocean
(a)
(b)
N
O
E
S
0
20 km
(c)
(d)
1.5 km

(6.66–6.07 and 6.06–5.56 m); the next stratum down was a transition zone that was emerged or submerged at a daily to weekly frequency (5.55–5.32 m), whereas the two lowermost zones were constantly wet, albeit under very shallow (5.31–5.06 m) to shallow (5.05–4.56 m) waters.

The first three hypotheses were tested by comparing total belowground, total aboveground, and species' biomass, respectively, before (1993–1994) and during the low-level conditions of 1999. A total of 197 vegetation quadrats from 13 fortnightly visits to the Boucherville wetland (6 May – 20 October 1999) were sampled. Maximum summer biomass (3 August – 3 September) estimates obtained in 1999 were compared with 93 quadrats sampled in 1993–1994 at Boucherville and three neighbouring wetlands (Pointe aux Trembles, Île aux Cerfeuils, and Verchères) using the same sampling method (Hudon 1997). Average water levels during the growth season (1 April – 30 September) of 1993 (7.06 m) and 1994 (6.79 m), for which comparable biomass data were available, were representative of the previous 10-year mean water levels (1983–1992 mean level ± SD = 6.74 ± 0.35 m).

Plant biomass was collected at 1- to 5-m intervals, depending on the slope, along a permanent transect line located perpendicular to the shoreline running from the upper dry grounds to a water depth of about 1 m. Samples were collected at defined field points located so as to cover small-scale changes occurring across the elevation gradient; for a given field elevation point, successive fortnightly samples were distanced laterally from the previous ones by about 25 cm. Plant biomass was assessed by collecting all plant matter (aboveground and belowground structures) in 25 cm × 25 cm quadrats placed at permanent markers at recorded intervals along a transect line (in shallow water). Vegetation growing in the quadrats was collected by hand using a shovel or a knife to loosen sediments and dislodge roots. Only the most commonly encountered species were accounted for in the present study, as biomass was sampled in small-size quadrats; the effects of water level variations on overall species diversity will be documented in a future paper.

For each quadrat, aboveground parts were identified and processed separately for each species, whereas belowground parts and litter were treated on a community basis. Periphyton, loose detritus, and sediment were washed away, after which plant matter was dried to a constant mass and weighed (0.001 g), leading to calculations of biomass of macrophyte dry mass (DM) (grams) per square metre. Emergent plant biomass was characterized separately for obligatory (*Schoenoplectus*, *Sagittaria*, *Butomus*, *Typha*, etc.) and facultative (*Polygonum*, *Lythrum*, and *Phalaris*) wetland taxa (Reed 1988; Gauthier 1997). Species were identified and designated according to the nomenclature used by Marie-Victorin (1995).

The fourth hypothesis dealt with the efficiency of four species of emergent macrophytes at monopolizing incident light. This was quantified by measuring the fraction of incident light (I_0) remaining at different heights above the ground within a dense, monospecific cluster of each species using a 4π probe (LI-COR SA-LI188B).

Statistical analysis

Differences in level, climatic conditions, frequency of occurrence, and biomass (ln transformed) observed before and during low water level episodes were tested by multiple Student's t tests (Fisher exact test, two-tailed). The biomass of individual species within a given stratum was compared, excluding samples of species that were not observed. Differences in the proportion of emergent and submerged vascular plants and of algae in the aboveground biomass within a given stratum were tested using multiple analyses of variance and Wilks' λ statistic. A bootstrap procedure was used to correct probability levels for all multiple tests (SAS version 8.0) (SAS Institute Inc., Cary, N.C.).

Results

Environmental conditions in 1931 and 1999

The growing seasons of 1931 and 1999 (1 April – 30 September) were characterized by significantly lower water levels and warmer air and water temperatures relative to the previous 10 years (Table 1). However, cumulated precipitation levels during both low water level episodes did not differ significantly from the previous 10-year period.

During the growing season, average St. Lawrence River water levels for the 1989–1998 period (6.59 m) were 85 cm higher than in 1999 (5.74 m) (Fig. 3*a*). A difference of 1.02 m was also observed between 1931 (6.09 m) and the previous 10 years (7.11 m). The low values reported in 1931 were 35 cm higher in 1999, although this includes an uncertainty related to historical changes in datum and measurement methods.

Between-year disparities in weather conditions and water levels further generated interannual differences in water temperature; in low-water years, waters were about 2.5 °C warmer on a monthly average basis (Table 1; Fig. 3*b*). The combination of low water levels and warm air and water temperatures resulted in conditions that differed consistently from the previous 10-year average conditions for wetland plants.

Effect of water levels on major species composition in 1931 and 1999

In comparing 1931 and 1999, compositional changes due to low water levels and changes that could be attributed to other environmental causes could be distinguished. This exercise revealed major similarities and also some differences in dominant species composition for the periods before and during the low water level conditions of 1931 and 1999; for the sake of clarity, current observations and those of Marie-Victorin (1943) were organized according to the elevation gradient of major riparian vegetation strata (Table 2): shrubs, meadow, marsh, barren zone, and open water.

The low water levels experienced in 1931 and 1999 brought about a marked, similar change in the species composition of all strata, except the upper shrubby zone. Meadows and marshy areas were invaded by *Phalaris arundinacea*, various grasses, and other facultative annual wetland plant species. Very shallow open waters previously colonized by submerged species became a dry, barren zone colonized by annual terrestrial plants (*Impatiens capensis* and *Polygonum* spp.), whereas waterlogged mudflats allowed for the germination and extensive flowering of *Alisma gramineum*.

If the species mentioned by Marie-Victorin (1943) are taken as commonly occurring, the comparison of major species composition between 1931 and 1999 reveals the lesser abundance of *Equisetum* spp., *Strophostyles helveola*, *Najas*

Table 1. Comparison of water levels and climatic conditions observed during the growing season (1 April – 30 September) for sampling years of low water levels (1931 and 1999) and the preceding 10-year period.

	1921–1930 (mean (SD))	1931 (mean)	1989–1998 (mean (SD))	1999 (mean)
N (years)	10	1	10	1
Water level (m)	7.11 (0.37)	6.09***	6.69 (0.38)	5.74***
Air temperature (average monthly) (°C)	15.2 (0.96)	17.0***	15.6 (0.71)	17.5***
Precipitation (cumulative monthly rain) (mm)	88.1 (15.40)	96.7 ns	84.5 (9.37)	81.6 ns
Water temperature (average monthly) (°C)	14.4 (1.00)	17.0***	15.3 (0.84)	17.8***

Note: The probability level of significant difference between years of low level and the preceding 10-year period (Student's *t* tests) is indicated as not significant (ns, $p > 0.05$) or very highly significant (***, $p < 0.001$).

Fig. 3. Seasonal variations in (*a*) water level and (*b*) water temperature in 1931 and 1999 compared with the previous 10-year intervals of 1921–1930 and 1989–1998 for the Montreal area. IGLD85, International Great Lakes Datum of 1985.

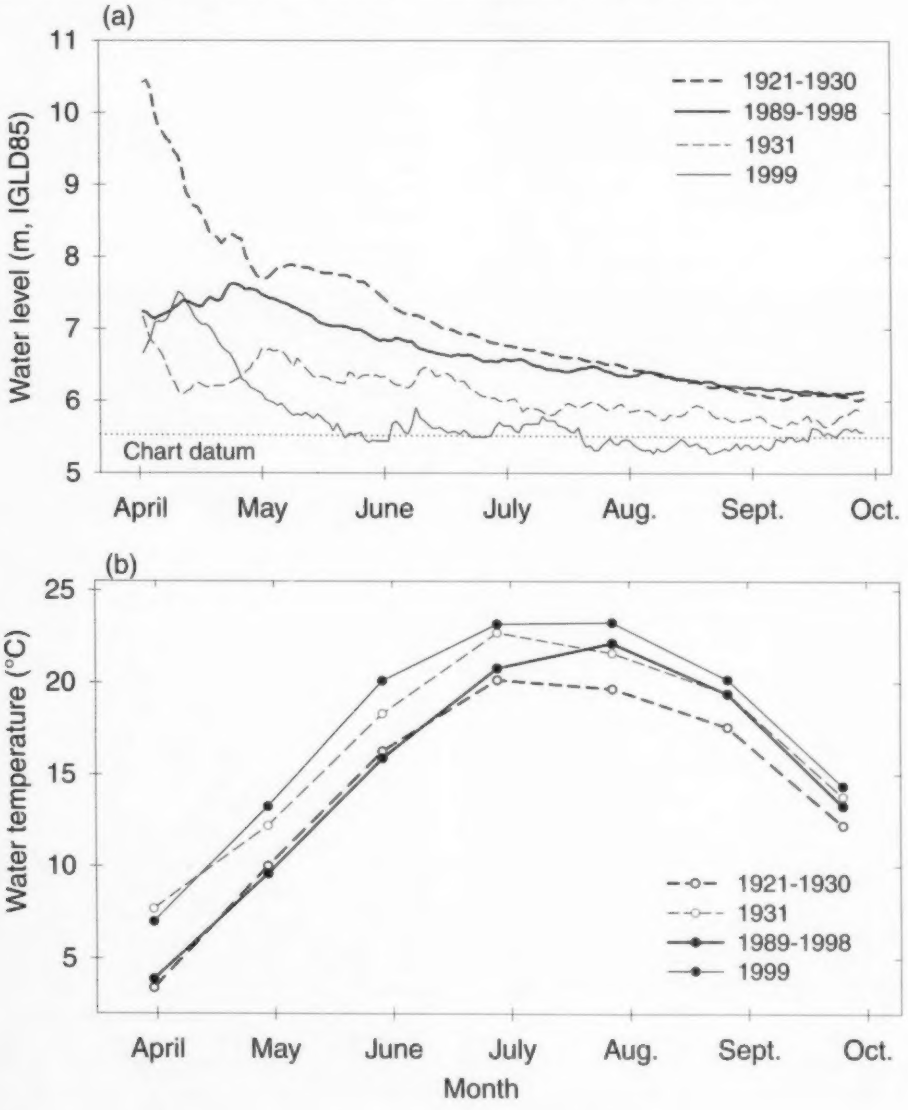


Table 2. Comparison of wetland strata and species composition between conditions reported before 1931 and before 1999 (this study, in bold) and low water level episodes documented in 1931 (Marie-Victorin 1943) and 1999 (this study, in bold) for the Boucherville Islands and Longueuil.

Stratum	Before 1931	**Before 1999**	1931	**1999**
Shrubs	*Salix interior* and *Salix longifolia* flooded extensively in spring due to ice jams and high water levels	***Salix* sp. abundant on higher grounds**	Extensive flowering of *Salix* due to rapid water level drop in early spring	**No observation of flowering conditions**
Wet meadow	*Plantago major*, *Xanthium pungens*, *Ambrosia artemisifolia*, *Strophostyles helveola*, *Equisetum arvense*	***Phragmites australis*, *Phalaris arundinacea*, *Butomus umbellatus*, *Equisetum fluviatile*, *Rumex verticillatus***	Wet meadow becomes dry. Proliferation of *Phalaris arundinacea*, which formed large colonies in subsequent years and later invaded the entire island. Persistence of *Equisetum arvense*	**Same as 1931. Proliferation of *Phalaris arundinacea* and *Phragmites australis***
Marsh	*Butomus umbellatus*, *Equisetum palustris* var. *americanum*, *Equisetum fluviatile*, *Equisetum littorale*	***Typha angustifolia*, *Phragmites australis*, *Lythrum salicaria*, *Schoenoplectus lacustris*, *Bolboschoenus fluviatilis*, *Sparganium eurycarpum*, *Sagittaria* sp. with submerged *Elodea canadensis*, *Myriophyllum* spp.**	Marsh turns into a meadow. Proliferation of *Phalaris arundinacea* and disappearance of *Equisetum* spp. Persistence of *Butomus umbellatus*	**Same as 1931. Proliferation of Graminea and facultative wetland (annual) species, various Compositae, and *Lythrum salicaria*. Reduction of obligatory wetland species. Disappearance of submerged species**
Very shallow open water	*Myriophyllum exalbescens*, *Elodea canadensis*, *Heteranthera dubia* (on mud or gravel bottom), *Najas flexilis*, *Callitriche hermaphroditica*	***Myriophyllum* spp., *Elodea canadensis*, *Heteranthera dubia*, *Potamogeton richardsonii*, *Stuckenia pectinata***	Dry, barren zone colonized by annuals such as *Polygonum lapathifolium* var. *nodosum*, *Cyperus inflexus*, *Cyperus esculentus*	**Same as 1931. *Polygonum* spp., *Cyperus* spp., *Populus deltoides***
Shallow open water	*Chara* spp. (on clay or mud bottom)	***Myriophyllum* spp., *Elodea canadensis*, *Heteranthera dubia*, *Vallisneria americana*, *Ceratophyllum demersum*, *Potamogeton richardsonii*, *Stuckenia pectinata***	Waterlogged mud extensively colonized by emergent and flowering *Alisma gramineum*. Disappearance of submerged plants (*Chara* spp.)	**Very shallow water colonized by *Chara* spp., dense filamentous algae, and submerged plant debris brought in by short-term rises in level**
Open water (0.3–3 m deep)	Presence of *Butomus umbellatus* var. *vallisneriifolius*, submerged sterile form with ribbon-like leaves	***Vallisneria americana*, *Elodea canadensis*, *Myriophyllum* spp., *Heteranthera dubia*, *Potamogeton richardsonii***	Shallow open water shows proliferation of *Butomus umbellatus* var. *vallisneriifolius* with ribbon-like leaves extending to and floating on the surface. Presence of the submerged, linear-leaved forms of *Sagittaria heterophylla* and *Alisma gramineum*	**Same as in 1931. Presence of submerged, linear forms of *Butomus* and *Alisma*. Proliferation of other submerged vascular plants, especially canopy-forming species**

flexilis, and *Callitriche hermaphroditica* and the increased occurrence of *Phragmites australis*, *Potamogeton crispus*, *Potamogeton richardsonii*, and *Stuckenia pectinata*, as well as filamentous algae, in the 1990s.

The similarities between our results and the general pattern observed in 1931 suggest that a closer examination of the overall zonation of wetland plant biomass and of its short-term seasonal changes would yield general information and allow the testing of hypotheses on the response mechanisms of this community under changing water levels.

Effect of water levels on vertical zonation of biomass

Under the water level conditions observed for the 1989–1998 period (6.59 m), the vertical zonation and species composition in St. Lawrence River wetlands reproduced the usual pattern, including wet meadows, emergent marshes, and submerged vegetation (Fig. 4*a*), as previously documented in numerous ecosystems. Under the level conditions of 1999, all land located at elevations above 5.56 m (chart datum level) was above the waterline and at elevations from 5.56 m down to 4.56 m was submerged under <1 m of water (Fig. 4*b*).

Interannual variations in water levels resulted in differences in the vertical distribution of biomass of primary producers along the elevation gradient, in terms of both quantity and quality of organic matter (Fig. 4), thus generating extremely different conditions for aquatic fauna. Under 1999 levels, the total aboveground biomass was nearly twice the amount recorded previously and followed a different distribution along the littoral zone. In the 1993–1994 period, aboveground biomass decreased progressively from about 1 to 0.38 kg $\mathrm{DM{\cdot}m^{-2}}$ as depth increased along the littoral area, whereas in 1999, biomass was discontinuous and showed higher values (1.67–0.63 kg $\mathrm{DM{\cdot}m^{-2}}$) at both ends of the elevation range (Fig. 4). An overall smaller total biomass was observed in 1993–1994, providing a continuous cover across the riparian zone and a greater quantity of food for obligatory aquatic organisms. The low water levels of 1999 shifted wetlands towards drier conditions, inducing a spatially discontinuous plant biomass dominated by Graminea and robust emergents, bearing cellulose-rich tissues most likely processed by terrestrial detritivores.

Plant response mechanisms to low water levels

Shallow marsh turned into dry meadow (6.66–6.07 m)

At this elevation, wetland plants growing in shallow (10–30 cm) water in previous years were dry for the entire summer of 1999. Families of perennial (Graminea and Lythracea) and annual (Renonculacea, Compositae, and Polygonaceae) facultative wetland plants formed the bulk (>50%) of total aboveground biomass in 1999, with most abundant taxa (*Phalaris arundinacea*, *Leersia oryzoides*, and *Polygonum* spp.) showing a significant ($p < 0.003$) rise in frequency of occurrence. Aboveground biomass was highest (>1.5 kg $\mathrm{DM{\cdot}m^{-2}}$) at this elevation; belowground biomass increased significantly in 1999 (Table 3), indicating the development of a thick root network to capture soil moisture.

Deep marsh turned into wet meadow (6.06–5.56 m)

Under 1993–1994 water level conditions, Boucherville and other wetlands in the Montreal area were dominated by obligatory wetland species such as *Sparganium eurycarpum*, *Bolboschoenus fluviatilis*, *Schoenoplectus* spp., *Butomus umbellatus*, and *Sagittaria latifolia* growing in water <1 m deep. Submerged plants (*Myriophyllum* spp., *Elodea canadensis*, and *Ceratophyllum demersum*) grew in the shallow waters among the rooted emergent macrophytes.

In 1999, the 1-m drop in water level pushed the water's edge about 70 m horizontally. Emergent and aquatic vegetation thus grew under mostly dry, sometimes waterlogged conditions, with brief periods of shallow water (2 cm) incursions during the summer of 1999 (Fig. 4*b*). As was observed in the previous elevation stratum, belowground and litter biomass increased significantly in 1999 (Table 3). Although total aboveground biomass did not differ significantly (Table 3), significant changes in the proportions of submerged and emergent wetland plants were observed (Table 4).

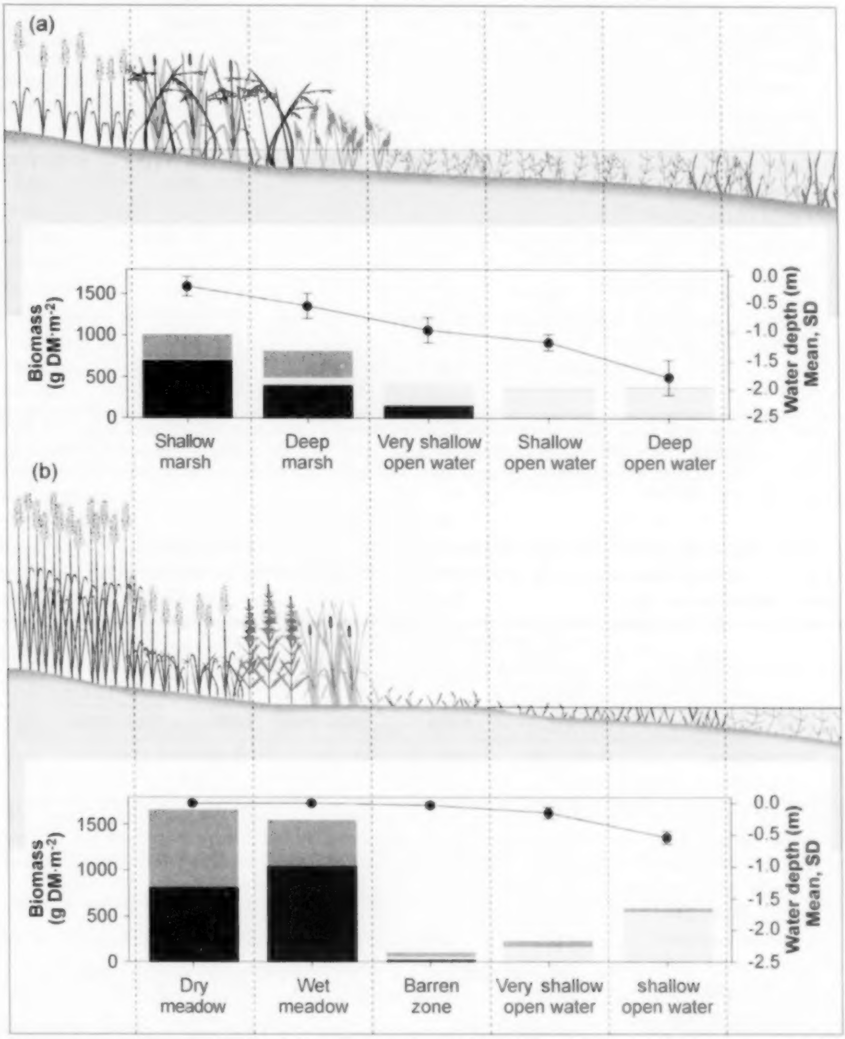
Submerged plants disappeared and the abundance of previously dominant obligatory plants decreased, whereas several grass (*Phalaris arundinacea*, *Phragmites australis*, and *Leerzia oryzoides*) and annual (*Impatiens capensis*, *Polygonum* spp., *Ranunculus trychophyllum*, *Sonchus oleraceus*, and *Hypericum* spp.) wetland species proliferated (Table 5). Submerged plant species, which used to grow among the stems of emergent plants under average level conditions, were mostly absent in 1999, except for small (<2 cm) *Heteranthera dubia* germlings, which were recorded significantly more often ($p < 0.001$) on waterlogged soil than in 1993–1994.

Obligatory emergent taxa (*Sagittaria latifolia*, *Schoenoplectus lacustris*, and *Schoenoplectus pungens*) also showed a significantly reduced frequency of occurrence in 1999. In 1993–1994, *Sagittaria latifolia* was the most common species at the outer edge of the band of emergent plants but it occurred significantly less often in 1999 ($p < 0.001$). This was attributed to the freezing of its belowground structures owing to the prevailing low water levels throughout the previous (1998–1999) winter, as supported by the observation of decaying belowground structures in early June 1999. The biomass of *Butomus umbellatus* also declined significantly in 1999 (Table 5).

Owing to its emergent, yet waterlogged soil conditions, devoid of the thick cover of space- and light-monopolizing *Phalaris*, this elevation stratum supported the highest number of species in 1999. Taxa occurring the most frequently under dry conditions were *Polygonum* spp. (62% of 47 samples), *Typha angustifolia* (55%), and *Bolboschoenus fluviatilis* (30%); *Lythrum salicaria* (either small germlings or adult plants) was observed in 9% of samples. *Lythrum salicaria* seedlings were especially numerous just above the water line (5.56–6.06 m) where open, dry ground was available for colonization in between shoots of obligatory emergent wetland species. By the end of August, small *Populus deltoides* grew at the lower edge of this zone in the dry, open area.

Exposure to light in 1999 was high, as annuals grew near the fringe of large, readily established obligatory emergents. Facultative wetland plant species also colonized the dry, exposed soil between obligatory wetland species using the light and space available between the scattered stems. Major differences were measured in the effectiveness of wetland plant species to monopolize incident light (Table 6). Plant morphology and biomass had a marked effect on light attenuation and ground-level illumination. Emergent plants with linear leaves and unramified stems, such as *Schoenoplectus*

Fig. 4. Schematic representation of wetland plant zonation observed (*a*) before and (*b*) during an episode of low water levels showing the limits of the elevation strata and corresponding changes in water depth and plant assemblages documented in this study. For each hydrological regime and plant community, the distribution of aboveground biomass is divided into emergent macrophytes (solid bars), submerged aquatic vegetation (light shaded bars), and litter (medium shaded bars). DM, dry mass.



lacustris, exhibited lower extinction coefficients per unit of biomass than *Typha angustifolia* and graminoid plants supporting a well-developed inflorescence at their apex. Germlings from the seed bank were thus exposed to 32% of incident light among stems of *Schoenoplectus lacustris*, 7% among *Typha*, and <1% of incident light among Graminea (*Phalaris arundinacea* and *Phragmites australis*).

Very shallow open water turned into barren ground (5.55–5.32 m)

At this elevation, the water was about 1-m deep under 1993–1994 conditions, resulting in a dense cover of submerged plants; under low water levels, this zone alternated between submerged (up to 30 cm in May and October) and dry (August–mid-September) conditions, as water levels fluctuated above and below 5.56 m (equivalent to chart datum level) during most of the summer of 1999 (Fig. 3*a*). In fact, this stratum was the most obviously impacted by low levels, as it supported the lowest biomass of all strata (Table 3) and appeared as a band of dried, barren soil with a dense layer of filamentous algae (Table 4) located below the threshold of previously established emergent macrophytes yet above the level that was constantly submerged. The rapid succession of flooded and dried-out conditions thus effectively prevented the establishment of either aquatic or terrestrial vegetation, as seedlings of both types were killed by the onset of new water level conditions. This resulted in a significant (fourfold) reduction in *Vallisneria americana* biomass (Table 5).

Shallow water turned into very shallow open water (5.31–5.06 m)

This stratum was always submerged, although average water depth decreased from about 1.2 to 0.2 m when water levels dropped in the summer of 1999; from mid-July to

Table 3. Comparison of late summer (late July – early September) aboveground, belowground, and litter biomass measured before (1993–1994) and during (1999) a low water level episode in the St. Lawrence River wetlands.

Vegetation stratum and water depth (m) (mean (SD, *N*))		Total aboveground biomass (g DM·m^{-2}) (mean (SD))		Total belowground biomass (g DM·m^{-2}) (mean (SD))		Litter biomass (g DM·m^{-2}) (mean (SD))	
1993–1994	1999	1993–1994	1999	1993–1994	1999	1993–1994	1999
Shallow marsh: 0.20 (0.17, 19)	Dry meadow: 0 (0, 20)	721 (497)	817 (628) ns	1586 (2086)	2310 (1296)*	298 (512)	848 (442)***
Deep marsh: 0.54 (0.22, 35)	Wet meadow: 0 (0, 13)	495 (371)	1046 (771) ns	490 (869)	1507 (1433)**	326 (544)	503 (456)*
Very shallow open water: 0.96 (0.22, 14)	Barren zone – waterlogged mud: 0.03 (0.04, 21)	430 (311)	60 (58)***	103 (143)	10 (11)**	< 1 (0)	51 (82)***
Shallow open water: 1.17 (0.14, 16)	Very shallow open water: 0.15 (0.09, 18)	370 (253)	228 (82) ns	50 (72)	25 (16) ns	7 (16)	7 (5)***
Deep open water: 1.78 (0.31, 21)	Shallow open water: 0.54 (0.10, 20)	362 (244)	592 (364) ns	41 (44)	47 (41) ns	6 (18)	37 (44)***
Overall weighted mean biomass		395.6	699.8	439.1	776.3	126.7	283.4

Note: The probability level of significant biomass difference between periods (Student's *t* tests on ln-transformed data), after correction for multiple tests, is indicated as follows: *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.001$); ns, not significant). The two median strata, which covered only a 25-cm elevation range, were assigned half the weight of the elevation strata covering a 0.5-m range for the calculation of overall weighted mean. DM, dry mass.

Table 4. Comparison of the proportion of total aboveground biomass found in emergent and submerged vascular plants and macroscopic algae measured in late summer (late July – early September) before (1993–1994) and during (1999) a low water level episode in the St. Lawrence River wetlands.

Vegetation stratum and water depth (m) (mean (SD, *N*))		% of emergent vascular plants (mean (SD))		% of submerged vascular plants (mean (SD))		% of macroscopic algae (mean (SD))		Difference in % among plant groups (*p*)
1993–1994	1999	1993–1994	1999	1993–1994	1999	1993–1994	1999	
Shallow marsh: 0.20 (0.17, 19)	Dry meadow: 0 (0, 20)	95 (14)	100 (0)	<1 (2)	0 (0)	5 (14)	0 (0)	0.17 ns
Deep marsh: 0.54 (0.22, 35)	Wet meadow: 0 (0, 13)	68 (42)	100 (0)	32 (42)	0 (0)	0 (0)	0 (0)	0.03*
Very shallow open water: 0.96 (0.22, 14)	Barren zone – waterlogged mud: 0.03 (0.04, 21)	31 (45)	35 (44)	69 (45)	17 (28)	0 (1)	48 (40)	0.00001***
Shallow open water: 1.17 (0.14, 16)	Very shallow open water: 0.15 (0.09, 18)	0 (0)	0 (0)	95 (15)	69 (24)	5 (15)	31 (24)	0.003**
Deep open water: 1.78 (0.31, 21)	Shallow open water: 0.54 (0.10, 20)	0 (0)	0 (0)	96 (13)	88 (23)	4 (13)	12 (23)	0.16 ns

Note: The probability and significance level of the difference in the percentage of plant groups between periods (Student's *t* tests on ln-transformed data), after correction for multiple tests, is indicated as follows: *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.001$); ns, not significant.

mid-September 1999, water depth was <0.10 m, with brief periods at <0.02 m. Submerged macrophytes (*Elodea canadensis*, 96% of 52 samples; *Vallisneria americana*, 85%; *Myriophyllum* spp., 96%; *Potamogeton* spp., 58%; and *Heteranthera dubia*, 58%) represented the largest proportion of aboveground biomass over both periods (Table 4). However, the water level drop in 1999 resulted in a markedly different species composition and biomass in that year (Fig. 4*b*), with a significant rise in the proportion of macroscopic algae (Table 5). *Chara* sp. (69% of samples) and filamentous algae (46% of samples) were observed significantly ($p < 0.001$) more often in 1999 than in 1993–1994. Proliferation of macroscopic algae most likely resulted from their ability to grow rapidly under conditions of high temperature, high illumination, and rapid depth fluctuations, including occasional, albeit short-lived, dry periods.

When the lowest water levels were reached in mid-August, bleached filamentous algae and *Chara* sp. formed a crust on top of vascular macrophytes, which remained alive throughout the midsummer low-water period. After 1 month of very low water levels (depth <0.10 m, late August), numerous germlings of *Typha angustifolia* and *Alisma gramineum* were observed, the latter forming extensive prairies with emergent flowering structures throughout adjacent shallow-water mudflats. Emergent macrophyte germlings disappeared after water depth rose above 0.25 m after mid-September.

Table 5. Comparison of late summer (late July – early September) biomass recorded for various macrophyte taxa collected in the same elevation range (4.56–6.66 m) in the period before (1993–1994) and during (1999) a low-water level episode in the St. Lawrence River (Montreal area).

Taxon	Wetland classification	1993–1994 biomass (g DM·m^{-2}) (mean (SD, *N*))	1999 biomass (g DM·m^{-2}) (mean (SD, *N*))	Difference in biomass (*p*)
No. of quadrats (*N*)		93	92	
Emergent vascular plants				
Populus deltoides	OBL	0	0.6 (0, 1)	—
Polygonum spp.	FAC	0	97.7 (164.3, 19)	—
Phalaris arundinacea	FAC	189.8 (199.2, 3)	520.7 (597.7, 19)	1 ns
Leersia oryzoides	OBL	0	30.6 (37.7, 12)	—
Other Graminea	—	0	1.20 (0.71, 4)	—
Lythrum salicaria	FAC	34.4 (0, 1)	481.8 (228.8, 3)	0.58 ns
Typha angustifolia	OBL	122.7 (0, 1)	434.4 (626.1, 23)	1 ns
Butomus umbellatus	OBL	313.9 (267.0, 7)	13.6 (14.4, 12)	0.04*
Sparganium eurycarpum	OBL	865.8 (481.5, 10)	124.3 (92.5,3)	0.06 ns
Bolboschoenus fluviatilis	OBL	376.0 (280.9, 6)	212.8 (211.4, 15)	0.94 ns
Schoenoplectus spp.	OBL	381.9 (354.8, 16)	13.8 (20.0, 13)	0.03*
Eleocharis smallii	OBL	24.4 (27.7, 8)	14.7 (22.9, 11)	1 ns
Sagittaria latifolia	OBL	368.8 (333.3, 26)	49.0 (30.4, 4)	0.49 ns
Alisma plantago-aquatica	OBL	43.4 (71.8, 7)	3.5 (3.8, 8)	0.92 ns
Ranunculus spp.	OBL	4.0 (0, 1)	27.5 (37.3, 5)	1 ns
Submerged vascular plants				
Ceratophyllum demersum	OBL	24.0 (57.5, 9)	15.6 (39.3, 8)	1 ns
Lemna trisulca	OBL	0	0.3 (0.2, 7)	—
Elodea canadensis	OBL	86.2 (143.3, 36)	102.7 (176.3, 43)	1 ns
Myriophyllum spp.	OBL	177.5 (239.6, 42)	67.5 (96.5, 43)	0.59 ns
Heteranthera dubia	OBL	29.1 (52.7, 10)	78.6 (137.6, 32)	0.76 ns
Potamogeton spp.[a]	OBL	49.6 (55.2,16)	27.5 (40.1, 20)	1 ns
Vallisneria americana	OBL	113.8 (95.3, 35)	27.2 (27.6, 40)	0.04*
Macroscopic algae				
Filamentous algae	OBL	70.9 (59.7, 3)	32.1 (34.8, 42)	1 ns
Nitella sp.	OBL	23.4 (43.0, 10)	0	—
Chara spp.	OBL	0	26.7 (41.3, 41)	—

Note: Taxa were classified into obligatory (OBL) and facultative (FAC) wetland plants (Reed 1988; Gauthier 1997). The probability of significant difference in frequency of occurrence and in biomass between periods (Student's *t* tests on ln-transformed data), after correction for multiple tests, is indicated as follows: *, significant ($p < 0.05$); ns, not significant. A dash indicates that the test could not be performed. DM, dry mass.

[a] Includes *Potamogeton richardsonii*, *Potamogeton crispus*, and *Stuckenia pectinata* (*Potamogeton pectinatus*).

Deep open water turned into shallow open water (5.05–4.56 m)

Under 1993–1994 water levels, this elevation range was beneath 1–2 m of water, yet in 1999, water depth was reduced to <1 m, with depth being shallowest (30 cm) in August. This stratum exhibited a significantly higher aboveground biomass in 1999 (Table 3), although the proportions of submerged vascular plants and macroscopic algae did not differ between periods (Table 4). A greater water depth than at the previous stratum fostered the formation of a two-layered assemblage, with species forming a subsurface canopy (*Elodea*, *Myriophyllum* spp., and *Potamogeton* spp.) expanding above those remaining closer to the bottom (*Vallisneria*). Throughout the sampling season, canopy-forming species represented >50% of total biomass, with maximum canopy development at the time of lowest water levels (August). Although the species composition and structural arrangement of the community were very similar for both periods, biomass of *Vallisneria americana* was significantly lower in 1999 (Table 5).

Discussion

Comparison of wetland responses to low levels in 1999 and 1931

Water level is the predominant physical force acting to shape wetland community structure and biomass, especially in the wet–dry transition zone, and this study offers the opportunity to quantify the magnitude and speed with which wetland species respond. Wetlands and riparian vegetation respond to small interannual changes in water levels (Keddy and Reznicek 1986). In addition to the low levels of the 1931 season, Marie-Victorin (1943) identified both the decrease in water levels in the 20 years previous (1910–1930) and the reduction in spring floods (as a result of the control of ice jams) as other hydrological mechanisms explaining the temporal changes in wetland vegetation.

Native and non-native species alike are reported to invade or increase in abundance along river margins following water level regulation (Johnson 1994; Bush and Smith 1995). Both Marie-Victorin's (1943) study and the present one

Table 6. Comparison of light extinction coefficient and % light intensity available for plant colonization among the stems of established strands of different emergent wetland species.

Taxon	Aboveground biomass (kg DM·m^{-2})	Plant height (H) (cm)	Light extinction coefficient (K) (m^{-1})	% incident light at ground level or below the canopy	Equation	r^2 (p)	N
Phalaris arundinacea	1	140	4.271	0.25	$I_z/I_0 = 0.0042e^{0.0427H}$	0.95 ($p < 0.001$)	15
Phragmites australis	1.5	250	2.607	0.15	$I_z/I_0 = 0.0031e^{0.0261H}$	0.95 ($p < 0.001$)	26
Typha angustifolia	1.5	200	1.326	7	$I_z/I_0 = -0.0135 + 0.052H$	0.94 ($p < 0.001$)	21
Schoenoplectus lacustris	0.1	120	0.941	32	$I_z/I_0 = 0.3258 + 0.0061H$	0.96 ($p < 0.001$)	13

Note: For each species, the equation allowing a determination of the fraction of incident light intensity (I_z/I_0) remaining within stands of different heights (H) is given, with the fraction of total variance explained (r^2), the probability level of the relation (p), and number of measurements (N). All relationships are very highly significant ($p < 0.001$). DM, dry mass.

highlight the success of taxa specifically adapted to water level variations, such as *Alisma gramineum* (on water-saturated mudflats) and *Butomus umbellatus* (submerged linear-leaved form). Rapid colonization by opportunistic annuals (*Polygonum lapathifolium*), perennials (*Cyperus* spp.), and trees (*Populus deltoides*) was also observed in both studies in the newly dried-out zone, as was the establishment of a new zonation based on a new water line (towards a new steady state). Plants with short generation times are often favoured in human-impacted ecosystems, but such species also benefit from the natural formation of disturbance patches (Grime 1979; Huston 1994).

In addition, both studies show the increased susceptibility to monospecific dominance by alien and aggressive species under low water levels: *Phalaris arundinacea*, *Butomus umbellatus* (in 1931 and in 1999), and *Phragmites australis* and *Lythrum salicaria* (in 1999 only). The abundance of *Butomus umbellatus* varied markedly since its introduction near Montreal in 1897. This species showed a strong expansion from 1920 to 1950, especially along the south shore of the Montreal archipelago, where it was the dominant plant on the littoral (Marie-Victorin 1943). Since the 1950s, this species' abundance and density have markedly decreased (Pageau et al. 1971), yet it remains common along St. Lawrence River shorelines. Whereas *Butomus umbellatus* turned out to be a lesser threat than originally anticipated by Marie-Victorin (1943, 1995), more recent invaders (especially *Phragmites australis* and *Phalaris arundinacae*) are likely to have a more pronounced impact on wetland diversity (Lavoie et al. 2003).

Major species composition also differed in some respects between 1931 and 1999. Marie-Victorin (1943, 1995) indicated that low-lying islands of the St. Lawrence River favoured many species of *Equisetum*, which formed distinct zones that included *Equisetum fluviatile*, *Equisetum arvense*, *Equisetum palustre* var. *americanum*, and *Equisetum littorale*. Although *Equisetum* spp. are still observed in more recent plant surveys, they no longer dominate riparian assemblages or form distinct zones; such a reduction in the abundance of Equisetales may be the result of generally drier conditions and (or) nutrient enrichment in water and sediments. The hypothesized shift in dominant species composition prompted by nutrient enrichment is supported by the results of a negative correlation of *Equisetum fluviatile* and of a positive correlation of *Typha angustifolia* with wetland fertility (Auclair et al. 1976).

Nutrient enrichment may also explain the occurrence, in the 1990s, of three species of *Potamogeton* (*Potamogeton crispus*, *Potamogeton richardsonii*, and *Stuckenia pectinata* (*Potamogeton pectinatus*)) tolerant to high nutrient concentrations (Riis and Sand-Jensen 2001), an event not mentioned in Marie-Victorin's (1943) paper. Eutrophication and warmer water temperatures may also account for the massive growth of *Chara* sp. and filamentous algae in 1999 in very shallow (<10 cm) waters, the latter also not reported by Marie-Victorin for 1931. Under low-level conditions, massive algal growth could be sustained by high light intensity, warm temperatures, and nutrients released from sediments and decomposing vascular plants in shallow water.

Wetland responses to low water levels

The adaptation of wetland plant taxa to water level variations largely depends on their ecological traits and on the period during which changes in levels occur, i.e., changes in biomass of algae, submerged vascular and emergent vascular plants were recorded over days, weeks, and months, respectively. Differences in weather and water level conditions between 1993–1994 and 1999 generated important differences in immersion–emersion regimes at equivalent elevations along the riparian zone, thus allowing the testing of the four hypotheses on the adaptations of wetland plants to a sharp drop in water level.

First, as hypothesized, the results of this study confirm that emergent plant assemblages adapt to low levels by increasing their belowground biomass, most likely as a response to a deepening of the water table (Tiner 1999). At the other end of the elevation range, the reduced occurrence of *Sagittaria latifolia* was shown to coincide with the freezing of its roots after low winter water level conditions.

Second, a sharp change in the proportion of total aboveground biomass was observed among emergent plants, submerged vascular plants, and macroscopic algae. Although total aboveground biomass did not change significantly for most elevation strata, wetland plants belonging to each group adjusted their biomass to current summer water level conditions. Dry conditions eliminated submerged vegetation at upper elevations and favoured macroscopic algae in the middle of the elevation range. Perennial obligatory wetland vegetation maintained itself under dry conditions for at least 1 year, thereby ensuring an intact emergent flora if water levels were to return to their previous values. Persistent changes in water level over many years can be accompanied by dramatic changes in vegetation, since it often takes plant communities 3–5 years to reestablish themselves in a wetland after a significant disturbance (Quinlan and Mulamootil 1987). Plant community displacement models with a constant topographic gradient are useful to assess changes in wetland zonation after interannual water level fluctuations (Herdendorf et al. 1981; Keddy and Reznicek 1986; Quinlan and Mulamootil 1987). The shift of vegetation to shallow or deep water can only occur if the slope is shallow, in the absence of natural or human-induced barriers, with suitable substrate, and if the rate of water level change is gradual enough for vegetation to reestablish itself (Bedford et al. 1976).

Third, a decrease in water levels coincided with a general tendency to less aboveground biomass in obligatory species and higher facultative species biomass. However, after results were corrected for multiple tests, this shift was statistically significant for only two (*Butomus umbellatus* and *Schoneplectus* spp.) of the 15 obligatory and none of the three facultative wetland taxa examined, owing to the small sample size and high variability of biomass of individual taxa. In spite of the general decrease in their aboveground biomass, deep-rooted perennial obligatory wetland species growing at high elevations did not die down and remained in place. Conversely, the biomass of the three facultative wetland taxa (*Polygonum* spp., *Lythrum salicaria*, and *Phalaris arundinacea*) tended to increase during the episode of low levels by factors of >100, 14, and 2.5, respectively, albeit not significantly. Although adult plants responded differently to low-level conditions, both facultative and obligatory emergent macrophyte species germinated in the barren, waterlogged mud zone. This observation suggests that given recurrent low levels, a dense cover of emergent vegetation could reestablish itself within 2–3 years.

Fourth, this study showed that invasive, aggressive plant species exhibited a higher capacity to monopolize light and space than less aggressive species. In decreasing order of their efficiency to monopolize light and space, dense strands of *Phragmites australis*, *Phalaris arundinacea*, and *Typha angustifolia* efficiently reduced ground-level light intensity to <10%, whereas *Schoenoplectus lacustris* allowed 32% light intensity at ground level. The aggressiveness of *Phragmites australis* and *Phalaris arundinacea* compared with other wetland taxa, including *Typha* spp., was also shown by Lavoie et al. (2003). Narrow-leaved native wetland species (*Typha angustifolia* and *Schoenoplectus lacustris*) allow a fraction of light to reach the ground–water surface, leading to the growth of some vascular plants and algae between their stems. Measurements of a 68–99.8% reduction of incident light within the canopy of various emergent wetland species are consistent with those reported by Ondok (1978). Such differences in strategic use of light and space by emergent species represent one of the mechanisms responsible for the establishment of different plant communities according to water level conditions.

Implications for faunal habitat quality

Water level variations affect the distribution and quality of biomass along the vertical shoreline gradient, as well as the overall productivity of the littoral fringe of wetlands, in terms of both primary and secondary production. Disruption of biomass cover and differences in carbon quality further alter the food chain structure supported by the wetland. It could be argued that episodes of low water levels increase the spatial variability of primary production, since such events favour high biomasses of cellulose-rich grasses and sedges at high elevations and a higher proportion of rapidly recycled labile macroscopic and filamentous algae at elevations located in the wet–dry transition zone. In the short term, low levels thus deprive aquatic fauna of the shelter provided by flooded, dense emergent plants and modify the distribution and type of food supplied by primary producers. Water level changes over a 5-year period resulted in an 18-fold difference in the primary productivity of a prairie wetland as the vegetation went through phases of drying, regenerating, degenerating, and flooding (van der Valk and Davis 1978).

Under 1993–1994 conditions, *Typha angustifolia*, *Schoenoplectus* spp., *Bolboschoenus fluviatilis*, and *Sagittaria latifolia* coexisted with *Elodea canadensis* and *Myriophyllum* spp. growing in cool, shaded waters among the stems, which provide food, shelter, and protection from predators for a wide variety of invertebrates and vertebrates. Flooded marshes with an optimal ratio of open water to emergent vegetation represent important habitats for aquatic birds and waterfowl (Weller and Spatcher 1965; Kaminski and Prince 1981), providing a food supply of plants and invertebrates, as well as shelter from predators (Lemay 1989). The presence of emergent plants shading the shallow waters from direct sunlight maintains cool water temperatures, prevents the excessive development of filamentous algae, and maintains high O_2 saturation.

Conversely, under 1999 conditions, the dense monospecific growth of *Phalaris arundinacea*, *Phragmites australis*, and *Lythrum salicaria* on dry ground monopolized most of the incident light and formed dense strands on dry land, thus preventing the growth of other plant species under its canopy. Although dense grass stands provide optimal nesting habitat for ducks, juvenile survival may be affected by the presence of a dry, barren zone at the water's edge; ducklings loafing or sleeping on mudflats and in open, dry areas are vulnerable to predation (Keith 1961) and disturbance.

Spring flooding of the tall grass cover that has proliferated during a season of low water levels in the previous summer may provide the optimal ground cover for esocid fish using the floodplain for egg laying and larval rearing (Casselman and Lewis 1996). *Phalaris arundinacea* and other graminoid plant cover is preferred to *Typha* spp. and *Schoenoplectus* spp., since it provides an elevated area for egg attachment and adequate cover for juveniles but little obstruction to adult movement (Franklin and Smith 1961; Fortin et al. 1982; Bry 1996). The benefits of alternating low summer levels and subsequent high spring flood for pike recruitment should be examined in the future, in conjunction with water levels and temperature effects within and outside the floodplain (Farrell 2000).

In the period before discharge regulation and control of ice jams (i.e., before 1960), repeated physical stresses induced by seasonal variations in water levels and ice kept the wetlands of the St. Lawrence River in a constant state of flux. Subsequent excavation of the Port of Montreal and shoreline alterations have likely reduced water circulation through the Boucherville archipelago, which is filling in with emergent vegetation and being invaded by new plant species. Such morphological alterations could explain some of the differences between the findings of Marie-Victorin (1943) and those of the present study.

Since 1960, human interventions such as water level regulation, reduction of ice scouring, control of ice jams, and reduction of spring floods have markedly decreased the within-year range of water levels but increased their between-year variability. Change in dominant species composition and biomass, proliferation of alien species, and addition of taxa tolerant to eutrophication are all indications of the major, cumulative, physical disturbances that the islands of Boucherville (and St. Lawrence River wetlands in general) have withstood over the decades. Whereas high water levels in Lake Ontario caused concern in the 1970s and 1980s, the 2000s may bring extended periods of low water levels in the Great Lakes. Although managing extremes is always a challenge, managing drought conditions may prove to be the biggest challenge of all, since drought exacerbates human pressures, thus imposing the need to weigh economic interests against other concerns. The strong response of St. Lawrence River wetlands to water level changes and the effects on the quality of faunal habitats make them highly vulnerable to the cumulative impacts of future climate change and human interventions.

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Diminished social status affects ionoregulation at the gills and kidney in rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Competition for social status can result in physiological differences between individuals, including differences in ionoregulatory ability. Subordinate rainbow trout (*Oncorhynchus mykiss*) had two-fold higher uptake rates of sodium across the gill and two-fold higher whole-body sodium efflux rates than the dominant fish with which they were paired. Sodium efflux was then divided into branchial and renal components, both of which were higher in subordinates. Branchial sodium efflux accounted for 95%–98% of sodium loss. Plasma sodium concentrations were more variable, although not significantly different, in subordinate fish, suggesting that the increased loss of sodium in these trout is compensated for by an increase in uptake rates. Urine flow rates and plasma cortisol concentrations were higher in subordinate fish, but there was no difference in glomerular filtration rate between dominants and subordinates. Renal sodium reabsorption was significantly reduced in subordinates. In summary, the ionoregulation of subordinate individuals was altered, most likely occurring as a result of stress-induced changes in gill permeability, resulting in a higher throughput of water and increased branchial sodium efflux. These changes in ionoregulatory ability have many physiological implications, including the increased susceptibility of subordinates to ionoregulatory challenges and an increased metabolic cost of ionoregulation.

Résumé : La compétition pour le statut social peut entraîner des différences physiologiques entre les individus, dont des différences dans la capacité d'ionorégulation. Les truites arc-en-ciel (*Oncorhynchus mykiss*) subordonnées ont une absorption deux fois plus grande de sodium par les branchies et des taux d'élimination du sodium au niveau du corps entier deux fois plus importants que les poissons dominants avec lesquelles elles sont appariées. Le sodium éliminé se divise en une composante branchiale et une composante rénale, qui sont toutes deux plus importantes chez les poissons subordonnées. L'élimination du sodium par les branchies représente 95 % à 98 % de la perte de sodium. Chez les poissons subordonnées, les concentrations plasmatiques de sodium sont plus variables, mais elles ne sont pas significativement différentes de celles des dominants, ce qui laisse croire que la perte accrue de sodium chez ces truites est compensée par des taux d'absorption plus élevées. Les taux d'écoulement de l'urine et les concentrations plasmatiques de cortisol sont plus élevés chez les poissons subordonnés, mais il n'y a pas de différences dans les taux de filtration glomérulaire entre les dominants et les subordonnés. La réabsorption de sodium par le rein est significativement réduite chez les subordonnés. En bref, l'ionorégulation des truites subordonnées est modifiée à cause très probablement de changements dans la perméabilité des branchies produits par le stress, ce qui entraîne un passage accru de l'eau et une élimination plus importante du sodium. Ces changements de la capacité ionorégulatrice ont plusieurs conséquences physiologiques, dont une susceptibilité accrue, chez les subordonnés, aux stress d'ionorégulation et un coût métabolique plus grand de l'ionorégulation.

[Traduit par la Rédaction]

Introduction

The stress encountered by a subordinate fish within a dominance hierarchy is sufficient to alter its physiology (reviewed by Sloman and Armstrong 2002). Recently, Sloman et al. (2002*a*) found that subordinate rainbow trout (*Oncorhynchus mykiss*) have higher branchial sodium uptake rates than their dominant counterparts and that this ionoregulatory disturbance can have implications for the accumulation of waterborne toxicants that enter via the sodium uptake path-

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way. However, the ionoregulatory differences that exist between dominant and subordinate animals are not clearly understood and it is not known whether this higher sodium uptake is driven by higher sodium efflux across the gills and (or) a difference in kidney function.

Salmonid fish form linear dominance hierarchies (Bachman 1984; Metcalfe et al. 1989) where position in the social hierarchy will denote both characteristic behaviours and physiological changes. These dominance hierarchies can be successfully replicated in the laboratory and when two fish are confined together, one fish will become dominant over the other. The present study aimed to examine sodium uptake and efflux and renal function in dominant and subordinate rainbow trout. Sodium crosses the apical membrane of the gill epithelia through a sodium channel driven by proton extrusion and is then transported across the basolateral membrane by Na^+/K^+-ATPase (Avella and Bornancin 1989; Lin and Randall 1995; Bury and Wood 1999). The stress response, i.e., to handling or confinement, is known to disturb sodium regulation (Mazeaud and Mazeaud 1981).

Loss of sodium is via two routes, branchial and renal. Sodium is lost by diffusion across the gills, accounting for approximately 90% of sodium loss at rest and even more during stress (see review by McDonald and Milligan 1997). Although usually a minor route of sodium efflux, sodium is also lost in the urine. In the majority of teleost fish, paired mesonephric ducts leave the kidney and unite in a structure known as the urinary bladder that serves as a functional extension of the kidney (Hickman and Trump 1969). Urine is stored in the urinary bladder for approximately 25–30 min before it is discharged via the urogenital papilla in periodic bursts (Curtis and Wood 1991). During this period of residence in the urinary bladder, ions can be reabsorbed to aid the kidney in salt conservation and this function of the urinary bladder may cut urinary losses of NaCl by at least 40% (Curtis and Wood 1991). Stress can also cause an increase in urine flow rate (diuresis) (Wood and Randall 1973; Vermette and Perry 1987), which along with decreased reabsorption of electrolytes in the kidney tubules and the urinary bladder can lead to an increased urinary sodium loss (Vermette and Perry 1987).

The present study aimed to identify and quantify any socially mediated differences in sodium balance at the gills and kidney as well as to assess the effects of social stress on kidney function using radioisotopic measurements of sodium turnover and renal processing. It was hypothesized that the chronic stress experienced by subordinate trout would severely impact upon ionoregulatory ability.

Materials and methods

Rainbow trout (weight 211.9 ± 6.8 g, fork length 26.7 ± 0.4 cm) were obtained from Humber Springs Trout Hatchery (Thamesford, Ont.) and held in 500-L stock tanks supplied with aerated, flowing, dechlorinated City of Hamilton tap water (hardness = 120 $mg \cdot L^{-1}$ as $CaCO_3$, $Na^+ = 0.6\ mmol \cdot L^{-1}$, $Cl^- = 0.7\ mmol \cdot L^{-1}$, $Ca^{2+} = 1.0\ mmol \cdot L^{-1}$, 12 °C, pH = 8.0). Fish were fed to satiation once daily with commercial trout food (Martin Mills Inc., Elmira, Ont.). Feeding was stopped 24 h prior to experiments. Three experiments were carried out to investigate sodium turnover in relation to social status. The first documented sodium uptake in dominant and subordinate fish. The second measured whole-body sodium excretion (branchial and renal combined). Finally, kidney function of dominant and subordinates was examined by measuring urinary sodium excretion, urinary flow rate (UFR), and glomerular filtration rate (GFR) using [1,2-^{3}H]-polyethylene glycol ([^{3}H]PEG-4000) as a marker for urination events. All experiments were carried out according to the guidelines of the Canadian Council for Animal Care.

Behavioural observations

During each observation period, the fish were scored according to three variables: position, colour, and food acquisition. Fish swimming in the water column scored three points, fish resting on the bottom of the tank scored two points, and fish swimming at the water surface scored one point. Swimming at the water surface is characteristic of subordinate fish (Sloman et al. 2000*a*). Where there was a colour difference between the pair of fish, the lighter fish scored one point and the other zero points, as darkening in colouration is indicative of subordinance (Keenleyside and Yamamoto 1962; O'Connor et al. 1999). Finally, during each behavioural observation, a single food item was introduced into the tank. The fish that acquired the food item scored one point and the other fish zero points. With the exception of this one food item (given three times a day at each behavioural observation), fish were not fed during the experiment to minimize the effect of sodium uptake from the diet. At the end of the experiment, the fish within each pair with the higher behavioural score was identified as the dominant fish.

Branchial sodium uptake rates

Eighteen rainbow trout were removed from the stock tank, anaesthetized in benzocaine (0.05 $mg \cdot mL^{-1}$), and individually marked with alcian blue dye injected into their fins (Kelly 1967). Initial fork lengths and weights were recorded and fish were allocated to size-matched pairs (mean size difference = 0.6 ± 0.1 cm), as used in many other behavioural studies (Abbott and Dill 1989; Øverli et al. 1999; Sloman et al. 2000*a*). Each pair of fish was placed in a 26-L plastic tank and individuals were separated from each other by an opaque plastic partition. The stocking density was therefore approximately 15 $g \cdot L^{-1}$, which is similar to other studies using an equivalent size of trout to examine social interactions (Sloman et al. 2002*b*). Following 24 h of acclimation to the tank, the plastic partitions were removed and the fish allowed to socially interact. Behavioural observations were made three times daily (0900–0930, 1300–1330, and 1700–1730) to determine the dominant and subordinate fish of each pair (Behavioural observations section). Sodium uptake was measured in the two fish simultaneously while social interaction continued. In detail, after 48 h of social interaction, the water flow to the tanks was stopped and ^{22}Na (1 $\mu Ci \cdot L^{-1}$ (37 kBq)) (NEN-Dupont, Boston, Mass.) was added to the tank. Water samples were taken 15 min and 4 h after the addition of the isotope and analysed for both ^{22}Na radioactivity and total sodium concentration using an 8-cm-well NaI crystal gamma counter (Canberra Packard MINAXI Auto

Gamma, 5000) and flame atomic absorption spectrophotometer (Varian AA-220), respectively.

Four hours after the addition of isotope, a lethal dose of benzocaine was added to the tank (0.5 mg·mL^{-1}), which killed the two fish within 3 min. The fish were then removed and placed in a "cold" displacement rinse of sodium (100 mM) to remove any surface-bound ^{22}Na. The whole fish were cut up and then homogenized separately in a blender and 10 preweighed plastic scintillation vials filled with homogenate. The vials were reweighed and then counted for ^{22}Na activity with the same method as for water samples. Uptake rates of sodium were calculated as

$$\text{Uptake} = [(a/b)/c]/d$$

where a is total counts per minute in the fish, b is mean specific activity of the water (activity per unit concentration), c is weight of the fish (grams), and d is duration of exposure (hours) (Grosell et al. 1998).

Whole-body sodium efflux

Nine pairs of size-matched fish (mean size difference = 0.7 ± 0.1 cm) were marked as outlined previously. Each pair was placed in a 26-L tank and the dominant and subordinate individual of each pair identified as in the previous experiment. However, it was not possible to measure sodium efflux separately in the two fish while social interaction continued. Instead, following 48 h of social interaction as before, the fish were removed from the tank and anaesthetized in a solution of benzocaine (0.05 mg·mL^{-1}). Each fish was then injected with a dose of 50 µCi of ^{22}Na in 0.5 mL of Cortland saline (Wolf 1963) into the caudal vein as a marker for the appearance of sodium in the water. Fish were then placed individually in 5-L black Perspex "flux" boxes (9 cm × 35 cm × 16 cm) (McDonald and Rogano 1986) and supplied with flowing, aerated City of Hamilton tap water for a 14-h recovery period.

Following the recovery period, the inflow to the flux boxes was stopped, although aeration was continued, and a 5-mL water sample was taken every hour for the following 8-h period. A regression analysis was carried out to calculate the rate of appearance of ^{22}Na in the water. At the end of the 8-h sampling period, a lethal dose of benzocaine was added to the flux boxes (0.5 mg·mL^{-1}) and the fish removed. A blood sample was immediately withdrawn by caudal venipuncture into a heparinized syringe and centrifuged at 13 000g for 2 min. Half of the plasma was placed in a vial for later analysis of ^{22}Na activity and the remainder frozen in liquid nitrogen for later analysis of plasma total sodium concentration.

^{22}Na activity was quantified in both water and plasma samples by counting in a gamma counter. Sodium concentrations in the water and plasma were determined by flame atomic absorption spectrophotometry. Whole-body sodium efflux rates were calculated as

$$\text{Efflux} = (a/b)/c$$

where a is the rate of appearance of sodium in the water (counts per minute per hour), b is the plasma specific activity (counts per minute per micromole), and c is the weight of the fish (kilograms).

Renal sodium excretion and kidney function

Twenty-two rainbow trout were removed from the stock tank, anaesthetized, and marked in the same way as before. Size-matched pairs of fish (mean size difference = 0.3 ± 0.1 cm) were then allocated to 26-L plastic tanks and the dominant and subordinate fish within each pair determined as in the previous experiment. The disturbance and time (at least 48 h needed for postsurgery recovery; Wood and Patrick 1994) involved in implantation of urinary catheters for direct collection of excreted urine (Curtis and Wood 1991) would likely have obliterated any effects of dominance or subordinance on renal function. Therefore, the indirect method of Curtis and Wood (1991) for assessing urinary excretion was employed.

In detail, after 48 h of social interaction, fish were drawn from the tank and rapidly anaesthetized in benzocaine (0.05 mg·mL^{-1}). Fish were then injected with a dose of 17 µCi of [^{3}H]PEG-4000 (New England Nuclear; half life ~12.3 years) in 0.66 mL of Cortland saline (Wolf 1963) into the caudal vein as a marker for GFR and for the appearance of urine in the water. Fish were then placed individually in 5-L black Perspex "flux" boxes and supplied with flowing, aerated City of Hamilton tap water for a 14-h recovery period.

Following the recovery period, the inflow to the flux boxes was stopped and a 5-mL water sample was taken every 5 min for the following 8-h period. At the end of the 8-h sampling period, a lethal dose of benzocaine was added to the tank (0.5 mg·mL^{-1}) and the fish removed and immediately sampled for blood and urine. The blood samples were taken by caudal venipuncture as before. Half of the plasma was placed in a scintillation vial for later analysis of [^{3}H]PEG-4000 radioactivity and the remainder was frozen in liquid nitrogen and stored at –80 °C for later analysis of plasma ions and cortisol. A urine spot sample was then taken using a 30-cm piece of Clay-Adams PE-60 tubing with one end heat-flared to 1.5 times its original diameter and the other attached to a 1-mL syringe. The flared end of the catheter was inserted through the urogenital papilla into the urinary bladder and a slight suction applied to drain the urinary bladder as completely as possible (Curtis and Wood 1991). The urine sample was divided in the same way as the plasma and stored for later analysis of ion concentrations and isotope activity.

[^{3}H]PEG-4000 activities were quantified in water, urine, and plasma samples by scintillation counting. Plasma and urine samples were diluted with nanopure water and 5 mL of sample added to 10 mL of ACS fluor (Amersham) and then counted in a scintillation counter (LKB Rackbeta 1217 counter). Tests demonstrated that quenching was constant. Plasma and urine ions were determined by flame atomic absorption spectrophotometry. Cortisol concentrations were analysed using a radioimmunoassay (ICN Pharmaceuticals).

[^{3}H]PEG-4000 activity in the water increased with intervening periods of relative consistency as seen in previous studies (Curtis and Wood 1991). These "steps" were interpreted as points of urination, which were considered to have occurred when there was an increase in at least 20 counts·min^{-1} in a 5 mL sample compared with the one taken 5 min earlier. Total [^{3}H]PEG-4000 activity in the water (A_w, in total counts per minute) was calculated at each time point by multiplying the measured [^{3}H]PEG-4000 activity by the flux box volume. Individual urination burst volumes (V_{burst}) were calculated for each step increase in counts as

$$V_{\mathrm{burst}} = ((A_{\mathrm{w}_n} - A_{\mathrm{w}_{n-1}})/A_{\mathrm{u}})/\mathrm{mass}$$

where A_{u} is the measured [^{3}H]PEG-4000 activity in the urine in counts·min^{-1}·mL^{-1}. The average urination burst volume and the frequency of urination bursts were then calculated for each fish.

UFR was calculated as

$$\mathrm{UFR} = \frac{\sum V_{\mathrm{burst}}}{\mathrm{time}}$$

and GFR was calculated as

$$\mathrm{GFR} = \frac{\mathrm{UFR} \times A_{\mathrm{u}}}{A_{\mathrm{p}}}$$

where A_{p} is the measured [^{3}H]PEG-4000 activity in the plasma in counts·min^{-1}·mL^{-1}. Extrarenal clearance rate (ECR) could then be estimated as

$$\mathrm{ECR} = \frac{A_{\mathrm{w}} - \left(\sum V_{\mathrm{burst}} \times A_{\mathrm{u}}\right)}{A_{\mathrm{p}} \times \mathrm{mass} \times \mathrm{time}}$$

where A_{w} (total counts·min^{-1}) is the total [^{3}H]PEG-4000 activity appearing in the water over the 8-h period. Calculation of ECR assumes that any appearance of [^{3}H]PEG-4000 that did not occur in the periodic "steps" represented extrarenal excretion.

Urinary excretion rates (U) of sodium and potassium (X) were then calculated according to McDonald and Wood (1998) using the following equation:

$$U_X = [X]_{\mathrm{u}} \times \mathrm{UFR}$$

where $[X]_{\mathrm{u}}$ is the concentration in the urine.

Clearance rates (C) were calculated as

$$C_X = \frac{[X]_{\mathrm{u}} \times \mathrm{UFR}}{[X]_{\mathrm{p}}}$$

where $[X]_{\mathrm{p}}$ was the measured plasma concentration. Finally, clearance ratios (CR$_X$) for both sodium and potassium were calculated, relating the clearance of these ions to the GFR (i.e., to the clearance of [^{3}H]PEG-4000) (McDonald and Wood 1998):

$$\mathrm{CR}_X = \frac{C_X}{\mathrm{GFR}}$$

The lower the clearance ratio, the higher the efficiency of reabsorption. For example, a CR$_X$ of 0.1 would represent 90% reabsorption of filtered X by the renal system. The clearance ratio of water was calculated as

$$\mathrm{CR}_{\mathrm{H_2O}} = \frac{\mathrm{UFR}}{\mathrm{GFR}}$$

Statistical analysis

Data are given as means ± SE of the mean. Physiological measurements were compared between pairs of fish using Wilcoxon signed ranks test. SPSS® software was used for statistical analyses and the limit of significance in all analyses was $p < 0.05$.

Results

Subordinate fish had a twofold higher branchial sodium uptake rate of 730 μequiv.·kg^{-1}·h^{-1} when compared with 390 μequiv.·kg^{-1}·h^{-1} in dominant fish ($p = 0.038$) (Fig. 1).

When total sodium loss was measured using injected ^{22}Na as a radiotracer, subordinate fish had a higher overall loss of sodium when compared with dominants, the values being –744 and –324 μequiv.·kg^{-1}·h^{-1}, respectively ($p = 0.008$) (Fig. 2*a*), which were very comparable with the difference in uptake rates (Fig. 1).

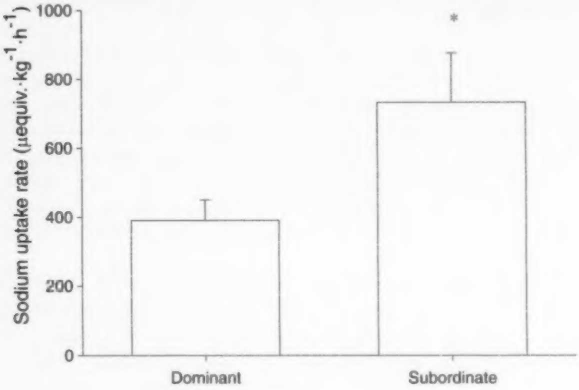
Subordinate fish in the experiments using injected [^{3}H]PEG-4000 to measure renal clearance had significantly higher plasma cortisol concentrations ($p = 0.012$) (Table 1) and significantly higher concentrations of sodium in their urine ($p = 0.021$) (Table 1). However, urine potassium levels and plasma ion levels were not significantly affected by social status (urine K$^+$, $p = 0.123$; plasma Na$^+$, $p = 0.081$; plasma K$^+$, $p = 0.177$; plasma Ca^{2+}, $p = 0.161$) (Table 1). There was a trend towards lower and more variable plasma sodium concentrations in subordinates, the values being 137.5 ± 5.3 μequiv.·L^{-1} in dominants and 111.7 ± 15.1 μequiv.·L^{-1} in subordinates (Table 1).

In general, urinary parameters were also more variable in subordinate fish. There were nonsignificant trends towards a higher average frequency and a higher average volume of urination bursts in subordinates (Table 1), which, in combination, resulted in significantly higher UFR in subordinate animals ($p = 0.012$) (Fig. 3*a*). Social rank did not affect GFR (Fig. 3*b*) or ECR (Table 1). Subordinate fish had significantly higher sodium (Fig. 2*b*) and potassium urinary excretion rates (Table 1) when compared with dominant fish (Na$^+$, $p = 0.018$; K$^+$, $p = 0.043$). Urinary sodium excretion rates were 31.7 μequiv.·kg^{-1}·h^{-1} in subordinates compared with 8.0 μequiv.·kg^{-1}·h^{-1} in dominant fish (Fig. 2*b*). Branchial sodium efflux was estimated (by subtraction of urinary sodium excretion rates from total sodium loss in the previous experiment) at 712 μequiv.·kg^{-1}·h^{-1} in subordinates and 316 μequiv.·kg^{-1}·h^{-1} in dominants (Fig. 2*c*). Subordinate trout also had significantly higher sodium clearance ratios when measured against the glomerular filtration marker [^{3}H]PEG-4000 (Na$^+$, $p = 0.018$) (Fig. 4), but potassium and water clearance ratios were not significantly different (Fig. 4). Sodium reabsorption efficiency by the renal system therefore fell from 99% in dominants to 87% in subordinates.

Discussion

The present study clearly illustrates that the branchial influx and efflux and renal clearance of sodium differ dramatically between subordinate and dominant animals. Firstly, subordinate animals demonstrate an elevation in sodium influx. Sodium influx rates for unstressed adult trout in Hamilton tap water are usually around 320 μequiv.·kg^{-1}·h^{-1} in fish of similar size (Goss and Wood 1990), which was approximately the same as seen in dominant fish (390 μequiv.·kg^{-1}·h^{-1}) of the present study. Higher sodium influx rates as a result of subordination have been illustrated previously in smaller fish (~5 cm)

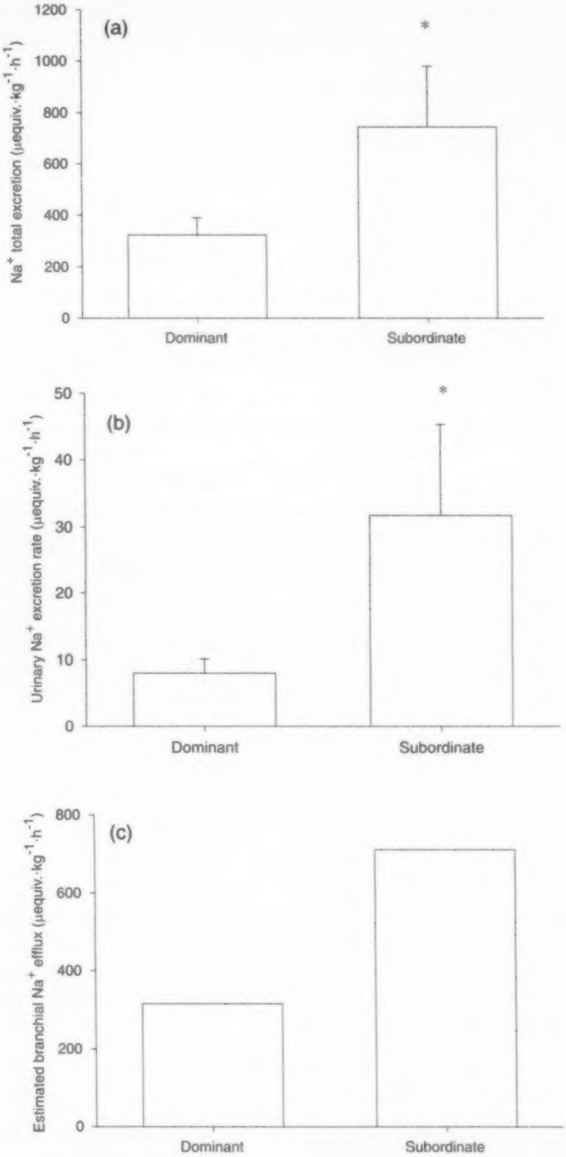
Fig. 1. Sodium uptake rates for dominant and subordinate fish measured using the radiotracer ^{22}Na. The asterisk denotes a significant difference ($Z = -2.073$, $p = 0.038$; $n = 9$ pairs of fish).



and have implications for the accumulation of toxicants that cross the gill via apical sodium channels (Sloman et al. 2002*a*). Total sodium efflux was comparably higher in subordinates when compared with dominants. The sodium influx and efflux calculations of the present study are based on different pairs of fish, but the effect of social status on sodium turnover appears relatively constant under these conditions with values for influx and efflux being comparable from both experiments.

Calculation of the urinary sodium excretion rate allowed for the division of total sodium loss into branchial and renal components. Urinary sodium excretion rates were higher in subordinates when compared with dominants, as was branchial sodium efflux. Urinary sodium excretion accounted for approximately 5% of sodium loss in subordinates and only 3% in dominant fish. Total sodium efflux rates of dominant fish were around 324 µequiv.·kg^{-1}·h^{-1}, which is comparable with sodium efflux rates of nonstressed fish of comparable size from previous studies (220 µequiv.·kg^{-1}·h^{-1}; Goss and Wood 1990). Urinary sodium excretion rates of nonstressed fish are approximately 5–8 µequiv.·kg^{-1}·h^{-1} (Curtis and Wood 1991), which is similar to the urinary excretion rate of dominant fish in the present study (8.0 µequiv.·kg^{-1}·h^{-1}). Plasma ions were not significantly different between dominant and subordinate fish, although there was a trend towards lower and more variable plasma sodium in subordinates. The greater loss of sodium from subordinates is counteracted to a variable degree by the higher sodium uptake rates.

Fig. 2. (*a*) Whole-body sodium loss, (*b*) sodium urinary excretion rates, and (*c*) estimated branchial sodium efflux (whole-body minus urinary excretion) for dominant and subordinate fish measured using ^{22}Na. The asterisks denote a significant difference (whole-body sodium loss, $Z = -2.666$, $p = 0.008$; sodium urinary excretion rates, $Z = -2.521$ $p = 0.012$; $n = 9$–11 pairs of fish).



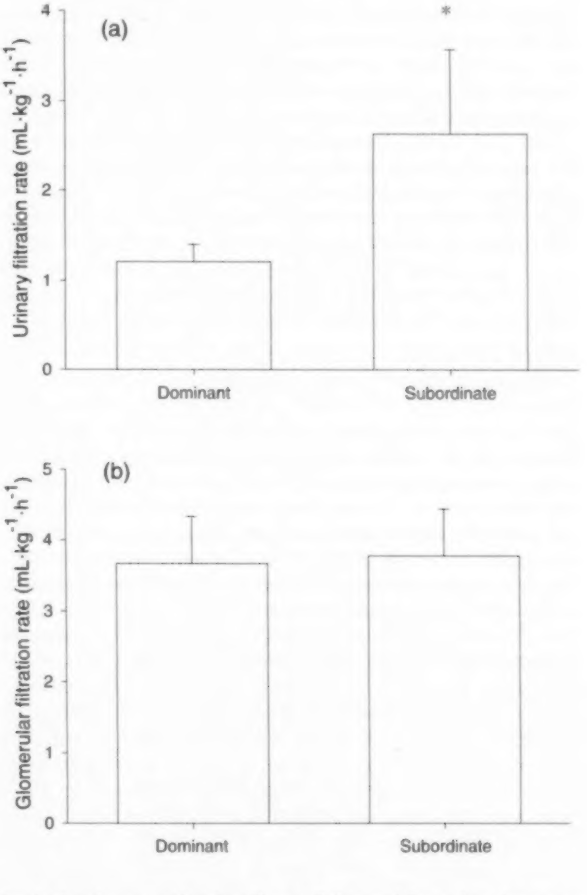
Branchial sodium efflux was estimated to be around 712 µequiv.·kg^{-1}·h^{-1} in subordinates compared with 316 µequiv.·kg^{-1}·h^{-1} in dominants. Subordinate fish also had a higher frequency and average volume of urination bursts, resulting in a significantly higher UFR, which is symptomatic of stress-induced diuresis (Wood and Randall 1973; Vermette and Perry 1987). Subordinate animals demonstrate many physiological changes indicative of a stress response (Pottinger and Pickering 1992; Øverli et al. 1999; Sloman et al. 2000*a*). The major hormones released during the stress response are catecholamines and cortisol. Significantly elevated concentrations of plasma cortisol have been widely documented in subordinate animals (reviewed by Sloman and Armstrong 2002), and in the present study, subordinate fish had higher plasma cortisol concentrations than their dominant counterparts. Plasma cortisol concentrations measured here are slightly higher than would be expected in unstressed fish (0–5 ng·mL^{-1}; Pickering and Pottinger 1989) but this is not surprising, as confinement in a flux box would act as an additional stressor. However,

Table 1. Plasma cortisol, urine and plasma ions, extrarenal [^{3}H]PEG-4000 clearance rates (ECR), and water clearance ratios for dominant and subordinate fish (n = 11 pairs of fish).

	Dominant	Subordinate	Statistical values
Plasma cortisol (ng·mL^{-1})	16.60±5.23	46.63±8.17	$Z = -2.521$, $p = 0.012$*
Urine Na$^+$ (mequiv.·L^{-1})	6.5±1.2	22.1±8.1	$Z = -2.310$, $p = 0.021$*
Urine K$^+$ (mequiv.·L^{-1})	0.16±0.01	0.4±0.1	$Z = -1.540$, $p = 0.123$
Plasma Na$^+$ (mequiv.·L^{-1})	137.5±5.3	111.7±15.1	$Z = -1.376$, $p = 0.169$
Plasma K$^+$ (mequiv.·L^{-1})	3.2±0.2	4.0±0.1	$Z = -1.459$, $p = 0.646$
Plasma Ca^{2+} (mequiv.·L^{-1})	2.6±0.1	2.3±0.2	$Z = -1.580$, $p = 0.114$
Average V_{burst} volume (mL·kg^{-1})	1.05±0.15	1.90±0.95	$Z = -0.840$, $p = 0.401$
V_{burst} frequency (h^{-1})	1.17±0.12	1.63±0.28	$Z = -1.825$, $p = 0.068$
ECR (mL·kg^{-1}·h^{-1})	2.7±1.3	1.5±0.4	$Z = -0.700$, $p = 0.484$
K$^+$ urinary excretion rate (µequiv.·kg^{-1}·h^{-1})	2.0±0.3	3.0±1.0	$Z = -2.023$, $p = 0.043$*

Note: Statistical values are for Wilcoxon signed ranks tests and data are given as means ± SE of the mean. Asterisks denote statistically significant differences.

Fig. 3. (*a*) Urinary filtration rate and (*b*) glomerular filtration rate (measured using [^{3}H]PEG-4000) of dominant and subordinate fish. Asterisks denote significant differences (urinary filtration rate, $Z = -2.521$, $p = 0.012$; glomerular filtration rate, $Z = -0.980$, $p = 0.327$; n = 11 pairs of fish).



both dominant and subordinate fish would receive the same level of stress and the fact that values of sodium influx and efflux rates are comparable from the two experiments, one using flux boxes and the other not, suggests that this additional stressor did not greatly affect the results. While no significant effect of social status on catecholamine secretion rates upon stimulation of the chromaffin cells in situ with acetylcholine has been demonstrated (Sloman et al. 2002*b*), catecholamine secretion in vivo during social interactions in fish has not been examined. However, social interactions are known to affect catecholamine levels in mammals (Von Holst 1998), and therefore, it is likely that social status will influence catecholamine concentrations in fish as well.

During stress, increased concentrations of catecholamines alter blood flow through the gills, increasing the area available for ion and gas transfer (Randall et al. 1972). This increases gill permeability, in particular by increasing intralamellar pressure (Gonzalez and McDonald 1992), and results in an increase in diffusional water influx and in passive branchial efflux of ions (McDonald and Milligan 1997). During stressful events, this increase in diffusional surface area aids in increased oxygen uptake but leads to an osmorespiratory compromise, as the surface area for passive ion loss is also increased (Randall et al. 1972). Cortisol may also play a role in stress-induced ionoregulatory changes. McDonald and Wood (2004) found that blocking glucocorticoid receptors in rainbow trout reduced UFR but that further increases in cortisol using implants of cortisol had no effect on UFR.

An increase in gill permeability of subordinate trout caused by stress-related hormonal changes would explain the large increase in branchial sodium efflux. Branchial sodium efflux accounted for 95%–98% of overall sodium loss in the present study. It is likely that increased branchial sodium loss is the major driving force behind the changes in ionoregulation seen in subordinate fish, resulting in a compensatory increase in sodium uptake. However, in addition, the sodium clearance ratio of the renal system was significantly higher in subordinates (0.132 compared with 0.018 in dominants). Therefore, subordinate fish have reduced reabsorption efficiency (87% versus 99%) in the renal system, reflecting a change in tubular and (or) urinary bladder reabsorption.

Increased levels of stress hormones would also cause an elevation of diffusional branchial water influx which, coupled with an increase in UFR, suggest a stress-related, increased throughput of water (Wood and Randall 1973; McDonald and Milligan 1997). The elevation of UFR in

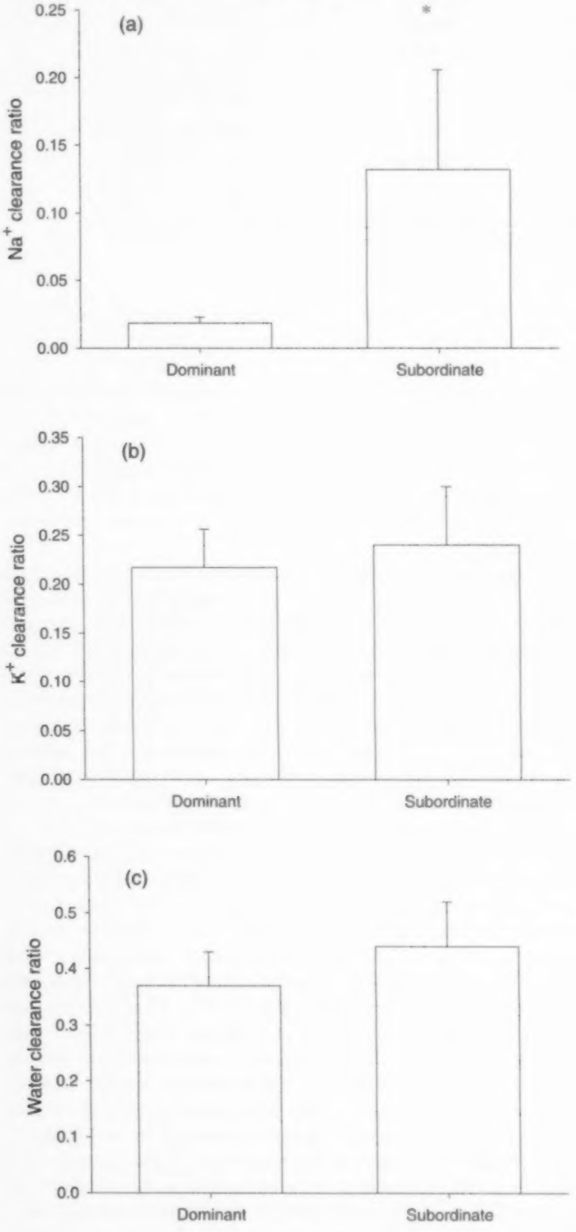


Fig. 4. (*a*) Sodium, (*b*) potassium, and (*c*) water clearance ratios of dominant and subordinate fish. Asterisks denote significant differences (Na$^+$ clearance ratio, $Z = -2.366$, $p = 0.018$; K$^+$ clearance ratio, $Z = -1.753$, $p = 0.080$; water clearance ratio, $Z = -0.314$, $p = 0.753$; $n = 11$ pairs of fish).

subordinate fish despite no change in GFR also suggests that the reduced reabsorption (i.e., increased excretion) of sodium and possibly other ions such as Cl$^-$ and K$^+$ increases water loss and hence elevates UFR. The trend towards a higher water clearance ratio in subordinate trout also supports the concept of increased throughput of water as seen in response to other stressors (McDonald and Milligan 1997).

The stress associated with subordination results in an increased branchial efflux of sodium. Urine flow rates are increased and there is a decreased reabsorption of sodium during renal processing. In combination, this increased loss of sodium drives the need for higher uptake of sodium across the gills and possibly the potential for increased sodium uptake from the diet (not measured in the present study). However, subordinate trout acquire a lower share of food among dominance hierarchies (Abbott and Dill 1989), and so, compensation by sodium uptake from the diet seems unlikely. Subordinate fish will also encounter an increased metabolic cost of ionoregulation associated with the increased active uptake of sodium (and potentially other ions such as Cl$^-$) from their freshwater environment. This is likely to be a contributing factor to the increased metabolic rate associated with subordinate trout following a social encounter (Sloman et al. 2000*b*), which would in turn affect the osmorespiratory compromise (Randall et al. 1972).

Many physiological consequences of social status have been documented in trout and in other animals (reviewed by Sloman and Armstrong 2002), and the present study increases our knowledge of the physiological differences that can exist between fish of different social status. While the social interactions occurring between pairs of fish may be structurally and socially simple compared with those occurring in the natural environment, it is well known that trout exhibit linear social hierarchies in field situations (Bachman 1984; Nakano 1995). Fish held in groups in larger laboratory stream tanks are generally found to exhibit socially mediated variations in physiology, qualitatively similar to those from studies of dyads (Sloman and Armstrong 2002). There are surprisingly few studies on the physiology of fish in relation to dominance hierarchies in natural habitats, but it is likely that many socially mediated differences in physiology found in the laboratory environment, such as those documented in the present study, will play a role in the natural environment.

The ionoregulatory stress, particularly with regard to sodium, seen in the subordinate animals of the present study can therefore have several important implications. Recently, Sloman et al. (2002*a*, 2003) demonstrated that higher sodium uptake rates in subordinate fish leads to greater uptake and accumulation of trace metal contaminants that cross the gill epithelia via the apical sodium channel (i.e., copper and silver). Sloman et al. (2002*a*) found socially mediated differences in copper uptake and accumulation both between pairs of fish and among groups of fish held in a stream tank environment. Animals whose ionoregulatory abilities are already compromised, such as subordinates, would likely have lower thresholds of effect for ionoregulatory toxicants, such as copper and silver (Laurén and McDonald 1985; Morgan et al. 1997). Certainly, under the conditions routinely used for toxicological assays, i.e., a small group of fish confined together in a lentic aquarium, individual differences in ionoregulation caused by the formation of dominance hierarchies have the potential to affect toxicity thresholds. Subordinate fish may also be at a disadvantage when encountering other ionoregulatory challenges, e.g., acclimation to ion-poor or acidic water (McDonald and Rogano 1986; Perry and Laurent 1989; Wood 2001).

In conclusion, subordinate fish display differences in sodium uptake and excretion from dominant animals, suggesting that the ionoregulatory ability of subordinate trout is compromised. There are significant implications for the fish in terms of physiological health, particularly during an ionoregulatory challenge such as acclimation to ion-deficient water or exposure to an ionoregulatory toxicant. Within populations of fish where strong dominance hierarchies exist, there is scope for the ionoregulatory abilities of individuals to vary markedly depending on their position in the social hierarchy.

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Evaluation of a chinook salmon (*Oncorhynchus tshawytscha*) bioenergetics model

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Abstract: We evaluated the Wisconsin bioenergetics model for chinook salmon (*Oncorhynchus tshawytscha*) in both the laboratory and the field. Chinook salmon in laboratory tanks were fed alewife (*Alosa pseudoharengus*), the predominant food of chinook salmon in Lake Michigan. Food consumption and growth by chinook salmon during the experiment were measured. To estimate the efficiency with which chinook salmon retain polychlorinated biphenyls (PCBs) from their food in the laboratory, PCB concentrations of the alewife and of the chinook salmon at both the beginning and end of the experiment were determined. Based on our laboratory evaluation, the bioenergetics model was furnishing unbiased estimates of food consumption by chinook salmon. Additionally, from the laboratory experiment, we calculated that chinook salmon retained 75% of the PCBs contained within their food. In an earlier study, assimilation rate of PCBs to chinook salmon from their food in Lake Michigan was estimated at 53%, thereby suggesting that the model was substantially overestimating food consumption by chinook salmon in Lake Michigan. However, we concluded that field performance of the model could not be accurately assessed because PCB assimilation efficiency is dependent on feeding rate, and feeding rate of chinook salmon was likely much lower in our laboratory tanks than in Lake Michigan.

Résumé : Nous avons évalué en laboratoire et en nature le modèle bioénergétique Wisconsin chez le saumon quinnat (*Oncorhynchus tshawytscha*). Des saumons quinnat gardés dans des aquariums de laboratoire ont été nourris de gaspareaux (*Alosa pseudoharengus*), leur nourriture principale au lac Michigan. Durant l'expérience, nous avons mesuré la consommation de nourriture et la croissance des saumons quinnat. Nous avons aussi dosé les concentrations de biphényles polychlorés (BPC) chez les gaspareaux et chez les saumons quinnat au début et à la fin de l'expérience afin d'estimer en laboratoire l'efficacité de la rétention des BPC par les saumons à partir de leur nourriture. D'après notre évaluation en laboratoire, le modèle bioénergétique procure des estimations justes de la consommation de nourriture chez le saumon quinnat. De plus, dans cette expérience de laboratoire, nous avons calculé que le saumon quinnat retient 75 % des BPC contenus dans leur nourriture. Dans une étude antérieure au lac Michigan, nous avions estimé à 53 % le taux d'assimilation des BPC par les saumons à partir de leur nourriture, ce qui laisse croire que le modèle surestimait considérablement la consommation de nourriture dans le lac. Toutefois, nous croyons que la performance en nature du modèle ne peut être vérifiée avec précision, parce que l'efficacité de l'assimilation des BPC est reliée au taux d'alimentation et que ce taux chez le saumon quinnat est vraisemblablement beaucoup plus bas dans les aquariums du laboratoire que dans le lac Michigan.

[Traduit par la Rédaction]

Introduction

Alewife (*Alosa pseudoharengus*) invaded Lake Michigan during the 1940s (Wells and McLain 1973). Declines in the abundance of several fishes, including yellow perch (*Perca flavescens*), deepwater sculpin (*Myoxocephalus thompsoni*), and emerald shiner (*Notropis atherinoides*), in Lake Michigan during the 1960s have been attributed to the alewife invasion. Alewives have been suspected of interfering with reproduction of these native fishes by feeding upon their pelagic fry (Wells and McLain 1973). Coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) were introduced into Lake Michigan during the 1960s to control the alewife population and to establish a sport fishery (Tody and Tanner 1966). Other salmonines, including rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush*), and brown trout (*Salmo trutta*), have been stocked into Lake Michigan since the 1960s as well. Stocking of salmonines appeared to be not only effective in reducing alewife biomass during the 1970s and early

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1980s but also effective in maintaining relatively low alewife biomass since the early 1980s (Madenjian et al. 2002*a*). The predominant predator on alewives in Lake Michigan has been chinook salmon. Similarly, chinook salmon has been the primary predator on alewives in Lake Huron (N. Dobiesz and J. Bence, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, U.S.A., personal communication) and Lake Ontario (Jones et al. 1993).

Bioenergetics modeling has served as a valuable management tool for Great Lakes fisheries (Stewart et al. 1981; Hansen et al. 1993; Jones et al. 1993). Bioenergetics modeling has been applied to salmonine populations to estimate lake-wide prey consumption. Comparison of the lake-wide prey consumption estimates with estimates of prey fish standing stock can be useful in regulation of stocking and harvest of salmonines.

Despite frequent application, the chinook salmon bioenergetics model developed by Stewart et al. (1981) and refined by Stewart and Ibarra (1991) has yet to be evaluated. Because chinook salmon is the predominant predator on alewives in three of the five Laurentian Great Lakes, information on the reliability of the chinook salmon bioenergetics model should improve the accuracy of estimates of uncertainty in decision models used to arrive at stocking and harvest rates of salmonines and ultimately lead to more effective fisheries management in these lakes. Further, should an evaluation of the chinook salmon bioenergetics model reveal inadequacies in the model predictions of consumption, then research could be initiated to correct the model and thereby improve the accuracy of model predictions of lake-wide prey consumption.

Madenjian et al. (2000) suggested a dual strategy to evaluate a fish bioenergetics model: (*i*) evaluate the model in the laboratory and (*ii*) use polychlorinated biphenyls (PCBs) as a tracer to evaluate the model in the field. The bioenergetics model can be evaluated in the laboratory by feeding fish a known amount of food, measuring growth, and then comparing observed food consumption with food consumption predicted by the model given the observed amount of growth (Whitledge and Hayward 1997; Madenjian and O'Connor 1999). In addition, during the laboratory evaluation, PCB concentration in the fish at the beginning and end of the experiment, as well as the PCB concentration in the food of the fish, can be determined. From these determinations, the efficiency with which the fish retain PCBs from their food can be calculated. Application of the bioenergetics model to estimate consumption in the field, coupled with PCB determinations of the predator and prey in the field, can generate a field estimate of the efficiency with which the predator retains PCBs from its food (Madenjian et al. 1998*a*). Comparison of the field and laboratory estimates of the efficiency with which the fish retains PCBs from its food provides an index of the accuracy of the bioenergetics model predictions of food consumption in the field (Madenjian et al. 2000).

The two-phase approach suggested by Madenjian et al. (2000) has useful diagnostic features. The laboratory evaluation serves as a check on the accuracy of the model's core processes, including respiration, egestion, and excretion. If the laboratory evaluation indicates significant bias in model predictions, then the fundamental equations for respiration, egestion, or excretion would need to be revised. However, if the laboratory evaluation indicates no significant bias in model predictions, then the field evaluation using PCBs as a tracer would provide a check on the accuracy of the specific parameters used to apply the model to the field; these specific parameters include the parameters used in the activity (swimming speed) submodel, the water temperature regime experienced by the fish in the field, and the predator and prey energy densities.

The objective of this study is to evaluate the Stewart and Ibarra (1991) chinook salmon bioenergetics model both in the laboratory and in the field using the approach suggested by Madenjian et al. (2000). The efficiency with which chinook salmon retain PCBs from their prey in Lake Michigan has been estimated (Madenjian et al. 2002*b*). This field estimate of net trophic transfer efficiency will be compared with our laboratory measurement of net trophic transfer efficiency in an attempt to gauge the accuracy of the bioenergetics model predictions of food consumption by chinook salmon in Lake Michigan.

Methods

Laboratory experiment

We ran our laboratory experiment from 6 June 2001 through 13 September 2001. Chinook salmon were obtained from the Fortsville – Lake George Fish Hatchery (Gansevoort, N.Y.) during November 2000, when mean total length of the chinook salmon was approximately 275 mm and the fish were 23 months old. These fish had been fed pelletized commercial trout food at the hatchery, and we continued to feed these fish the same diet in our laboratory at the Great Lakes Science Center from November 2000 through March 2001. Beginning in April 2001, the chinook salmon were acclimated to a diet of alewife. Alewife has been the predominant prey of age-1 and older chinook salmon in Lake Michigan (Stewart and Ibarra 1991; Madenjian et al. 2002*a*).

Chinook salmon were maintained in iron-filtered well water at the Great Lakes Science Center in four 2380-L circular fiberglass tanks that had a water exchange rate of 15 $L \cdot min^{-1}$. Average water velocities for tanks 1, 2, 3, and 4 were 20.0, 19.9, 19.3, and 26.4 $cm \cdot s^{-1}$ based on readings at 12 different locations in each tank (to yield an overall average water velocity for each tank). Water temperature was monitored daily in each of the four tanks; water temperature ranged from 10.7 to 13.0 °C and averaged 11.7 °C over the course of the experiment. The ambient well water temperature was chosen for the experiment because it approximated 11 °C, the water temperature at which Stewart and Ibarra (1991) assumed that age-1 and older chinook salmon in Lake Michigan would occupy during the summer. Photoperiod was controlled with fluorescent lighting, which was adjusted seasonally to mimic the duration of daylight for the Great Lakes region.

Tanks 1, 2, 3, and 4 received lots of 25, 26, 30, and 30 chinook salmon. Each chinook salmon was weighed on 6 June 2001 at the start of the experiment, on 11 July 2001, on 14 August 2001, and on 13 September 2001 at the end of the experiment. Chinook salmon were fed thawed alewives, which had been caught in Lake Michigan during November 2000 and March 2001, frozen, and stored at −30 °C. We attempted to maintain chinook salmon in tanks 1 and 2 on a ration of 2% of their body weight per day and chinook salmon in tanks 3 and 4 on a ration of 3% of their body weight per day. These rations were chosen because application of the bioenergetics model to chinook salmon in Lake Michigan indicated that chinook salmon, of similar size to those in the laboratory tanks, fed at a rate of about 3% of their body weight per day in Lake Michigan (Madenjian et al. 2002*b*). However, even though these rations were well below the maximum consumption levels as determined by the Stewart et al. (1981) bioenergetics model, the chinook salmon would not feed at these rates. We therefore adapted the feeding regime by offering the chinook salmon as much food as they would consume during one feeding each day. All uneaten food was removed, air-dried for 20 min, and weighed to the nearest 0.1 g. Averaging over the duration of the entire 99-day experiment, chinook salmon from tanks 1 and 2 consumed 1.4% of their body weight each day, and chinook salmon from tanks 3 and 4 consumed 1.6% of their body weight per day.

At the start of the experiment, 10, 11, 15, and 15 chinook salmon were sacrificed from tanks 1, 2, 3, and 4, respectively. The sacrificed fish were stored in plastic bags at –30 °C until time of analysis. All 15 of the remaining fish in each tank at the conclusion of the experiment were frozen at –30 °C until time of analysis. Additionally, five 50-fish subsamples of alewives caught in November 2000 and five 50-fish subsamples of alewives caught in March 2001 were stored at –30 °C for later analysis. To determine energy density, chinook salmon were composited by stage (start or end) of experiment and tank. Each chinook salmon composite, as well as each alewife subsample, was homogenized in a blender. A 1-g portion of each mixture was freeze-dried for 24 h; energy density was determined with a Parr model 1261 isoperibol calorimeter.

Bioenergetics modeling

A bioenergetics model for chinook salmon was originally formulated by Stewart (1980) and Stewart et al. (1981) and later refined by Stewart and Ibarra (1991). This model is one of a suite of fish bioenergetics models commonly referred to as Wisconsin bioenergetics models because most models were developed by researchers at the University of Wisconsin. We applied the updated version of the Wisconsin bioenergetics model for chinook salmon to the growth and consumption data from our laboratory experiment. Inputs to the model included (*i*) water temperature regime experienced by chinook salmon in our laboratory tanks, (*ii*) energy densities of the alewife fed to the chinook salmon, (*iii*) diet composition, on a wet weight basis, of the chinook salmon during the experiment, and (*iv*) energy densities of the chinook salmon during the experiment. Thus, our application differed slightly from the Stewart and Ibarra (1991) application to Lake Michigan chinook salmon. Rather than estimating the energy density of chinook salmon as a function of chinook salmon weight, per Stewart and Ibarra (1991), we inputted the initial and final energy densities of chinook salmon, by tank, into the bioenergetics model. We linearly interpolated the predator energy density over time between the start and completion of the experiment. In addition, we assumed that the chinook salmon just maintained their position within the water flow of the tank; the same type of assumption was made by Madenjian and O'Connor (1999) in their laboratory evaluation of a lake trout bioenergetics model. This assumption appeared to be reasonable, based on visual observations of fish in the tanks at various times of the day. Thus, for bioenergetics modeling purposes, we simulated chinook salmon at a constant swimming speed equal to the average flow rate within the tank.

Following the procedure of Madenjian and O'Connor (1999), we used the bioenergetics model in two ways: (*i*) to predict consumption given the observed starting and ending average weights of the chinook salmon over time t and (*ii*) to predict growth given the starting average weight of the chinook salmon and the observed average consumption over time t. Predictions were generated for each test period (time interval t of about 1 month) and for the entire duration of the experiment (time interval t of 99 days). All predictions were generated on a tank-by-tank basis. All bioenergetics modeling was performed using the most recent version of computer software for the Wisconsin bioenergetics model (Hanson et al. 1997).

Evaluation of bioenergetics model predictions

We evaluated the predictions of the chinook salmon bioenergetics model in a manner similar to that used by Madenjian and O'Connor (1999) to evaluate a lake trout bioenergetics model in the laboratory. For monthly predictions, we used a t test for paired comparisons to determine if the average difference between observed and predicted values was significantly different from 0. An average difference that was significantly different from 0 would indicate significant bias in the model predictions. In each case, the predicted value was subtracted from the observed value. Second, we performed simple linear regression analysis for the predicted values as a function of observed values. Under the condition that the model predictions were unbiased, the slope of this regression line would not differ significantly from 1, and the intercept of this regression line would not differ significantly from 0. We constructed Bonferroni 95% joint confidence intervals to test the null hypothesis that the slope was equal to 1 and the intercept was equal to 0 (Neter et al. 1983). These statistical analyses were applied to the sets of observations and model predictions for both consumption and chinook salmon weight.

Small sample size ($N = 4$) precluded formal statistical testing of the 99-day predictions of the bioenergetics model. Therefore, to compare predicted cumulative consumption with observed cumulative consumption, we simply formed the ratio of predicted cumulative consumption to observed cumulative consumption. Similarly, we compared the prediction of chinook salmon final weight over the entire 99-day period to the observed final weight by calculating the ratio of predicted value to observed value.

PCB determinations

Frozen chinook salmon were thawed and grouped by tank and stage of experiment (start or end). Each grouping of fish was then homogenized in an industrial-strength mixer. Each of the ten 50-fish subsamples of alewives was homogenized in a food processor.

The procedure outlined by Schmidt (1997) was followed to determine concentrations of 81 different PCB congeners in each of the homogenates. The detection limit for the PCB congeners ranged from 0.025 to 0.00001 $\mu g \cdot g^{-1}$. Total PCB concentration was determined by summing the concentrations of all 81 PCB congeners. Appropriate quality control samples (blanks, matrix spikes, and duplicates) were used to ensure the accuracy and precision of the samples. Spike recoveries ranged from 95% to 100%. All PCB concentrations are reported on a wet weight basis.

Calculation of net trophic transfer efficiency, γ, of PCBs

We calculated the efficiency with which chinook salmon retained PCBs from their food, γ, in the same manner as calculated by Madenjian et al. (2000):

$$(1) \qquad \gamma = \frac{\Delta\text{PCB body burden}}{\text{amount of PCBs ingested}}$$

where ΔPCB body burden is the average increase in the PCB body burden of chinook salmon in the tank during the course of the experiment (in micrograms of PCB) and amount of PCBs ingested is the average weight of PCBs in the food eaten by chinook salmon in the tank during the course of the experiment (in micrograms of PCB). We shall also refer to γ as the net trophic transfer efficiency of PCBs to chinook salmon from their food. Increase in PCB body burden was calculated as

$$(2) \qquad \Delta\text{PCB body burden} = ([\text{PCB}_\text{f}]W_\text{f}) - ([\text{PCB}_\text{i}]W_\text{i})$$

where $[\text{PCB}_\text{f}]$ is the average PCB concentration of chinook salmon in the tank at the end of the experiment (in micrograms per gram wet weight), W_f is average weight of chinook salmon in the tank at the end of the experiment (in grams wet weight), $[\text{PCB}_\text{i}]$ is the average PCB concentration of chinook salmon in the tank at the start of the experiment (in micrograms per gram wet weight), and W_i is the average weight of chinook salmon in the tank at the start of the experiment (in grams wet weight). To estimate the amount of PCBs ingested from the batch of alewives caught during November 2000, the PCB concentration of alewives caught during November 2000 was multiplied by the cumulative weight of November-captured alewives eaten by an average chinook salmon in the tank during the course of the experiment. A similar calculation was made to estimate the amount of PCBs ingested from the batch of alewives caught during March 2001. Summing the amount of PCBs ingested from the November-captured alewives and the amount of PCBs ingested from the March-captured alewives yielded the total amount of PCBs ingested by an average chinook salmon in the tank during the course of the experiment. PCB concentrations of November-caught and March-caught alewives were estimated by averaging the PCB concentrations of the appropriate five subsamples.

Field estimate of net trophic transfer efficiency of PCBs

Estimates of γ for Lake Michigan chinook salmon of ages 1–5 were 0.54, 0.59, 0.56, 0.50, and 0.47, respectively (Madenjian et al. 2002*b*). Averaging these estimates yielded a mean γ of 0.531. Madenjian et al. (2002*b*) used the updated version of the Wisconsin bioenergetics model for chinook salmon in deriving these estimates.

Comparison of laboratory and field estimates of γ

We used a two-sample *t* test to determine whether the mean laboratory estimate of γ was significantly different from the mean field estimate of γ. To estimate the degree of bias in the bioenergetics model predictions of food consumption in the field, we calculated the inverse of the ratio of the mean field estimate of γ to the mean laboratory estimate of γ (Madenjian et al. 2000).

Results

Laboratory evaluation of the model

Caloric density of chinook salmon decreased slightly in tanks 1, 2, and 3 during the course of the experiment, whereas the caloric density of chinook salmon increased slightly in tank 4 during the experiment. Initial chinook salmon caloric densities in tanks 1, 2, 3, and 4 were 5948, 6132, 6189, and 5938 $J \cdot g^{-1}$ (wet weight basis), respectively. Final chinook salmon caloric densities in tanks 1, 2, 3, and 4 were 5862, 5828, 5847, and 5943 $J \cdot g^{-1}$, respectively. Caloric densities of the five subsamples of November-caught alewives ranged from 5814 to 6141 $J \cdot g^{-1}$, with a mean and standard error of 6004 and 55 $J \cdot g^{-1}$, respectively. Caloric densities of the five subsamples of March-caught alewives ranged from 4829 to 5089 $J \cdot g^{-1}$, with a mean and standard error of 5050 and 49 $J \cdot g^{-1}$, respectively.

No bias was detected in the model predictions of monthly consumption. Results from a paired *t* test revealed that the mean difference between observed monthly consumption and predicted monthly consumption was not significantly different from 0 (mean difference = 0.8 g; $t = 0.11$, df = 11, $P = 0.9109$). Further, the slope of the regression line of predicted monthly consumption as a function of observed monthly consumption was not significantly different from 1 (estimate of slope = 0.80, estimate of Bonferroni joint 95% confidence interval half-width = 0.27), and the intercept of this regression line was not significantly different from 0 (estimate of intercept = 57.9 g, estimate of Bonferroni joint 95% confidence interval half-width = 78.5 g) (Fig. 1).

Monthly predictions of chinook salmon final weight were not significantly biased. Observed chinook salmon weight at the end of the test period was not significantly different from the weight predicted by the bioenergetics model (mean difference = –0.4 g; $t = -0.11$, df = 11, $P = 0.9156$). Additionally, the slope of the regression line of chinook salmon weight at the end of the test period predicted by the bioenergetics model as a function of observed chinook salmon weight at the end of the test period was not significantly different from 1 (estimate of slope = 0.95, estimate of Bonferroni joint 95% confidence interval half-width = 0.12), and the intercept of this regression line was not significantly different from 0 (estimate of intercept = 32.9 g; estimate of Bonferroni joint 95% confidence interval half-width = 73.8 g) (Fig. 2).

Bioenergetics model predictions of cumulative consumption over the 99-day experiment were within 8% of observed cumulative consumption (Table 1). Moreover, predicted cumulative consumption was within 2.5% of observed cumulative consumption for tanks 1, 2, and 4. Bioenergetics model predictions of chinook salmon final weight over the 99-day

Fig. 1. Predicted versus observed consumption by an average chinook salmon in a test tank (four tanks used in the laboratory experiment) during a test period (three periods per tank). Each test period was about 1 month long. Predictions were based on the bioenergetics model, developed by Stewart and Ibarra (1991), applied to each combination of tank and test period. The solid line represents the regression line fitted to the points. The broken line represents the line of 1:1 correspondence between predictions and observations.

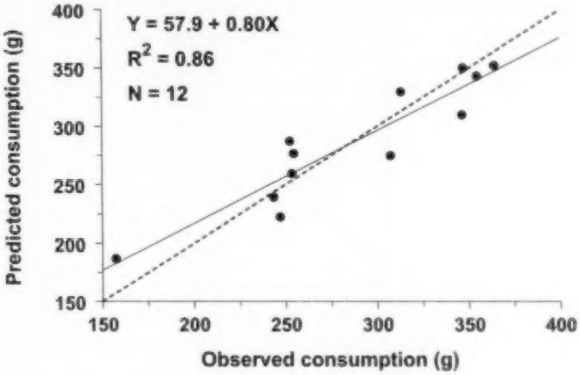
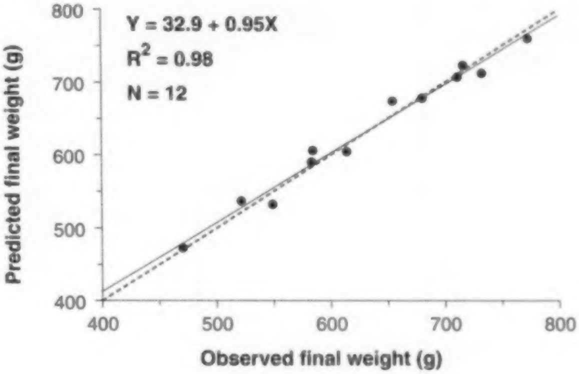


Fig. 2. Predicted versus observed final weight of an average chinook salmon in a test tank (four tanks used in the laboratory experiment) at the end of a test period (three periods per tank). Each test period was about 1 month long. Predictions were based on the bioenergetics model, developed by Stewart and Ibarra (1991), applied to each combination of tank and test period. The solid line represents the regression line fitted to the points. The broken line represents the line of 1:1 correspondence between predictions and observations.



experiment were within 5% of observed chinook salmon final weights (Table 1).

Field evaluation of the model using PCBs as a tracer

Initial PCB concentrations of chinook salmon composites ranged from 0.11 to 0.14 $\mu g \cdot g^{-1}$ (Table 2). Final PCB concentrations of chinook salmon composites ranged from 0.30 to 0.33 $\mu g \cdot g^{-1}$. Chinook salmon from tank 2 attained the greatest weight by the end of the experiment, whereas chinook salmon from tank 1 exhibited the smallest weight at the end of the experiment (Table 2). The amount of PCBs ingested was greatest for chinook salmon from tank 4 and lowest for chinook salmon from tank 1 (Table 2).

Average PCB concentration of the November-caught alewives that were fed to the chinook salmon was 0.25 $\mu g \cdot g^{-1}$, with the standard error of the mean equal to 0.01 $\mu g \cdot g^{-1}$. Mean PCB concentration for March-caught alewives was 0.30 $\mu g \cdot g^{-1}$, with the standard error of the mean equal to 0.01 $\mu g \cdot g^{-1}$. November-caught alewife PCB concentrations ranged from 0.22 to 0.27 $\mu g \cdot g^{-1}$, and March-caught alewife PCB concentrations ranged from 0.29 to 0.32 $\mu g \cdot g^{-1}$.

In the laboratory, net trophic transfer efficiencies of PCBs to chinook salmon from their food were 0.75, 0.83, 0.71, and 0.70 for tanks 1, 2, 3, and 4, respectively (Table 2). Thus, the mean laboratory estimate of γ was 0.75. The 95% confidence interval about this mean value ranged from 0.65 to 0.85. The mean value of γ from Lake Michigan field data was 0.53. The mean value of γ from the laboratory tanks was significantly greater than the mean value of γ from the Lake Michigan field data (t test, $t = 5.88$, df = 7, $P = 0.0006$).

Presenting the mean field and the mean laboratory estimates of γ to the nearest one thousandth, the field estimate was 0.531, whereas the laboratory estimate was 0.747. Based on these two estimates of γ, the chinook salmon bioenergetics model would appear to be overestimating food consumption by chinook salmon in Lake Michigan by 41%.

Discussion

Laboratory performance of the Wisconsin bioenergetics model for chinook salmon was satisfactory. The model furnished unbiased estimates of consumption and growth by chinook salmon in laboratory tanks. The respiration component of the chinook salmon bioenergetics model was based on oxygen consumption measurements for rainbow trout rather than chinook salmon (Stewart 1980). Thus, our laboratory experiment results corroborated the contention that rainbow trout metabolism was a suitable surrogate for chinook salmon metabolism. For three of the four laboratory tanks, predicted consumption of alewives by chinook salmon was within 2.5% of observed consumption over the 99-day experiment; and predicted consumption was within 8% of observed consumption for the fourth tank. Fishery managers would consider this level of accuracy to be acceptable for most management applications (J. Johnson, Michigan Department of Natural Resources, 160 East Fletcher Avenue, Alpena, MI 49707, U.S.A., personal communication; M. Staggs, Wisconsin Department of Natural Resources, P.O. Box 7921, Madison, WI 53707, U.S.A., personal communication).

Although comparison of the field and laboratory estimates of γ suggested that the Wisconsin bioenergetics model was substantially overestimating food consumption by chinook salmon in Lake Michigan, we concluded that field performance of the model could not be accurately assessed from our study. Daily food consumption by the chinook salmon in our laboratory experiment averaged about 1.5% of the salmons' body weight per day, but two pieces of evidence indicated that food consumption of similarly sized salmon in Lake Michigan was substantially higher than 1.5% of the body weight per day. First, application of the bioenergetics model to Lake Michigan chinook salmon yielded estimates

Table 1. Observed and predicted cumulative consumption and cumulative growth by chinook salmon in laboratory tanks.

Tank No.	Observed average weight of chinook salmon (g)		Observed consumption (g)	Observed GGE	Predicted consumption (g)	Predicted final weight (g)	Ratio of predicted to observed consumption	Ratio of predicted to observed final weight
	Initial	Final						
1	400	549	648	0.230	643	551	0.992	1.003
2	510	773	923	0.286	944	762	1.023	0.986
3	485	712	908	0.250	837	748	0.922	1.051
4	471	733	964	0.272	982	724	1.018	0.987

Note: The experiment ran 99 days. Predictions were based on the chinook salmon bioenergetics model developed by Stewart and Ibarra (1991). Chinook salmon were fed alewives. Observed consumption was equal to the total food eaten by all of the chinook salmon in a tank divided by the number of chinook salmon in the tank. Gross growth efficiency (GGE) was the gain in weight by the chinook salmon divided by the amount of food consumed.

Table 2. Estimation of γ, the efficiency with which chinook salmon retain PCBs from their food, from a laboratory experiment.

Tank No.	Average initial weight of chinook salmon (g)	Average final weight of chinook salmon (g)	Average initial [PCB] of chinook salmon ($\mu g \cdot g^{-1}$)	Average final [PCB] of chinook salmon ($\mu g \cdot g^{-1}$)	Consumption (g)	Amount of PCBs ingested (μg)	γ
1	400	549	0.111	0.302	648	162	0.748
2	510	773	0.123	0.332	923	233	0.834
3	485	712	0.144	0.326	908	229	0.708
4	471	733	0.135	0.318	964	242	0.699

Note: The experiment ran 99 days. Chinook salmon were fed alewives. Consumption was equal to the total amount of food eaten by all of the chinook salmon in a tank divided by the number of chinook salmon in the tank. Amount of PCBs ingested was equal to the total amount of PCBs ingested by all of the chinook salmon in the tank divided by the number of chinook salmon in the tank. Refer to eqs. 1 and 2 for more details on the calculation of γ.

of daily ration of similarly sized salmon approaching 3% of the body weight per day (Madenjian et al. 2002*b*). Second, chinook salmon gained on average only 2.3 g daily in our laboratory tanks, whereas average daily weight gain of similarly sized chinook salmon in Lake Michigan was 4.7 g (Madenjian et al. 2002*b*). Thus, a comparison of observed growth in the laboratory with that in Lake Michigan also suggested that food consumption rate was substantially higher in Lake Michigan than in our laboratory tanks. Based on laboratory experiments, PCB assimilation efficiency by lake trout was shown to decrease from 91% to 71% as food consumption rate increased from 0.5% of the body weight per day to the ad libitum ration; ad libitum ration averaged about 1.4% of the body weight per day for age-3 lake trout and about 0.9% of the body weight per day for age-6 lake trout (Madenjian and O'Connor 1999; Madenjian et al. 2000). If chinook salmon experienced a similar-sized decrease in their PCB assimilation efficiency as feeding rate increased from 1.5% to 3.0% of the body weight per day, then our laboratory estimate of γ would not be directly applicable to Lake Michigan chinook salmon.

In contrast with our chinook salmon bioenergetics model evaluation, it appeared that Madenjian and O'Connor (1999) were able to feed lake trout in laboratory tanks at a higher rate than that of lake trout in Lake Michigan. As mentioned above, age-3 and age-6 lake trout fed ad libitum while consuming 1.4% and 0.9% of their body weight per day, whereas similarly sized lake trout from Lake Michigan fed at 1.0% and 0.7%, respectively, of their body weight per day according to a bioenergetics model application to the Lake Michigan lake trout population (Madenjian and O'Connor 1999). Further, these apparent feeding rates in the lake were intermediate between the 0.5% of body weight per day and ad libitum rations administered in the laboratory experiment. Similarly, observed growth rates of lake trout in Lake Michigan were intermediate between the observed growth rates of similarly sized lake trout fed 0.5% of their body weight per day and ad libitum rations in the laboratory experiment (Madenjian et al. 1998*b*; Madenjian and O'Connor 1999). Both the laboratory and field evaluations of the Wisconsin bioenergetics model for lake trout indicated that the model was furnishing reasonably accurate predictions of food consumption by lake trout in Lake Michigan (Madenjian et al. 2000).

Just as chinook salmon in our laboratory tanks would not feed at as high a rate as chinook salmon in Lake Michigan, so too would coho salmon in the laboratory experiment conducted by Edsall et al. (1974) not feed at as high a rate as coho salmon in Lake Michigan. Edsall et al. (1974) generated an estimate of maximum consumption, $C_{\max}$, by measuring food consumption by coho salmon fed ad libitum in 870-L laboratory tanks. Stewart (1980) found that by using the Edsall et al. (1974) estimate of $C_{\max}$ in his preliminary bioenergetics model applications to coho and chinook salmon in Lake Michigan, both coho and chinook salmon required greater than "maximum" rations to achieve the observed growth rates. Consequently, Stewart (1980) revised the estimation of $C_{\max}$ using data from laboratory observations on sockeye salmon (*Oncorhynchus nerka*). Perhaps the appetite of coho and chinook salmon was somewhat suppressed by confinement in the laboratory tanks. However, it still may be possible to match the feeding and growth rates of Lake Michigan salmon in the laboratory if the salmon are kept in much larger tanks than the tanks used in our experiment.

Regardless of whether or not additional studies are undertaken to evaluate the Wisconsin bioenergetics model for chinook salmon, we see a potential need to revise the application of the model to Lake Michigan chinook salmon in the near future because recent observations have revealed a gap in our understanding of chinook salmon ecology in the Great Lakes. Preliminary data from archival tagging of Lake Huron chinook salmon with water temperature recorders suggested that Stewart and Ibarra (1991) may have underestimated summer temperatures experienced by chinook salmon. Whereas Stewart and Ibarra (1991) assumed that chinook salmon in Lake Michigan would remain in 11 °C water during the summer, recent data from the archival tags in Lake Huron chinook salmon indicated that average summer water temperature experienced by chinook salmon falls in the 13–17 °C range (R. Argyle, U.S. Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, U.S.A., personal communication). As more data become available from this archival tagging project, a new water temperature regime for chinook salmon can be constructed and then used in application of the bioenergetics model to chinook salmon in the lake. Additionally, based on recent determinations of lipid content in Lake Michigan chinook salmon, Stewart and Ibarra (1991) overestimated caloric density of Lake Michigan chinook salmon in their bioenergetics model application. Stewart and Ibarra (1991) assumed that lipid content increased from 5% to 20% as the chinook salmon increased in weight from 0 to 10 kg and then calculated chinook salmon energy density based on assumed lipid content. However, recent determinations for Lake Michigan chinook salmon indicated that average summer lipid content was only about 10% for chinook salmon greater than 1.2 kg in weight (A. Peters, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, U.S.A., personal communication). These recent lipid determinations are part of an ongoing project focusing on chinook salmon energy dynamics in Lake Michigan. Upon completion, this project should provide sufficient information to refine the predator energy density component of the bioenergetics model. Interestingly, according to some exploratory bioenergetics model simulations, the overestimation of chinook salmon energy density compensates for the underestimation of summer water temperatures occupied by chinook salmon; the net result is that consumption estimates from the newly revised exploratory model are within 5% of consumption estimates from the Stewart and Ibarra (1991) model. Nevertheless, we recommend that the chinook salmon model be revised once these ongoing studies are completed so that model applications to Great Lakes chinook salmon populations are more realistic.

Given the recent findings from the archival tagging project mentioned above, further laboratory evaluation of the chinook salmon bioenergetics model may be warranted. Bioenergetics models may not perform equally well at all water temperatures (Hartman and Brandt 1993). Thus, laboratory testing at water temperatures in the 13–17 °C range would be needed to complete the laboratory evaluation. Similarly, bioenergetics model performance may vary depending on fish size and ration (Bajer et al. 2003; Whitledge et al. 2003), and therefore, testing at additional sizes and rations would also be needed to fully evaluate the model.

Our laboratory estimate of γ for chinook salmon of 0.75 was not in accord with laboratory estimates of γ for coho salmon and rainbow trout. Gruger et al. (1975) fed juvenile coho salmon in laboratory tanks a pelletized commercial feed impregnated with a known concentration of PCBs, and their estimates of γ ranged from 0.30 to 0.60. Similarly, Gobas et al. (1999) fed pelletized commercial feed impregnated with a known concentration of PCBs to rainbow trout in laboratory tanks, and their estimate of γ was 0.48. Feeding rates were less than 1.5% of body weight per day in both the Gruger et al. (1975) and Gobas et al. (1999) studies. Chinook salmon, coho salmon, and rainbow trout all belong to the genus *Oncorhynchus*, and therefore, these three species would be expected to have similar efficiencies for retaining PCBs from their food. Perhaps the efficiency with which *Oncorhynchus* fish assimilate PCBs from their food is considerably lower on a commercial pellet diet than on an alewife diet. Additionally, there may leaching associated with pellets whereby PCBs are lost from the pellet after submersion in water but before consumption by fish (Reinert et al. 1974).

Our study has exposed the following limitation: to conduct a valid evaluation of a fish bioenergetics model in the field using PCBs as a tracer, average feeding rate in the laboratory tanks should be similar to the feeding rate in the field. As pointed out by Madenjian et al. (2000), each technique to evaluate fish bioenergetics models has advantages and disadvantages, and it is only through application of these evaluation techniques that weaknesses and limitations in the various techniques become uncovered. We suspect that trophic transfer efficiencies to fish from their prey of microcontaminants other than PCBs are also dependent on the feeding rate of fish. Long-term feeding and growth laboratory experiments with fish fed natural foods, in a manner similar to that used by Madenjian et al. (2000), would be needed to confirm this hypothesis.

Our study represents an important step in evaluating the chinook salmon bioenergetics model because the results from the laboratory evaluation indicated that the Stewart and Ibarra (1991) model was predicting chinook salmon consumption and growth in the laboratory without significant bias. Thus, our results provided the first indication that rainbow trout metabolism was an adequate substitute for chinook salmon metabolism. Further, our findings would be useful in diagnosing potential sources of error in generating model predictions for the field. Given no apparent bias in model predictions of consumption and growth in the laboratory, a field test revealing inadequacies in model predictions for chinook salmon in Lake Michigan would likely implicate an inaccurate swimming speed submodel. As previously mentioned, the error in characterizing chinook salmon energy density in Lake Michigan appeared to "cancel out" the error in characterizing the water temperature regime experienced by chinook salmon in Lake Michigan. Prey energy densities used in the model application to Lake Michigan chinook salmon were based on actual determinations on alewives from Lake Michigan in spring, summer, and fall during the 1970s and early 1980s (Stewart et al. 1981), and therefore, these energy densities were certainly representative of energy densities of the prey of chinook salmon in Lake Michigan. Thus, of the four groups of parameters specific to the model application to Lake Michigan chinook salmon (including the swimming speed submodel, the water temperature regime experienced by chinook salmon in Lake Michigan, predator energy density, and prey energy density), the swimming speed submodel group remains the only untested group.

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Survey gear calibration independent of spatial fish distribution

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Abstract: Trawl surveys provide important information for evaluation of relative stock abundance fluctuations over time. Therefore, when survey gears or vessels are changed, it is important to compare the efficiency and selectivity of old and new gears and vessels. A method for estimation of conversion factors is developed based on a survey design where paired hauls are taken in the same trawl track line. The method explicitly accounts for changes in fish density caused by trawling disturbance. A generalized linear model for paired hauls catches is analytically derived and the gear conversion and disturbance parameters with their precision are obtained using standard software. Simulation studies carried out additionally showed that the estimated conversion factors were practically unbiased. Because of the independence of the spatial fish distribution, the new method is preferable to the traditional paired hauls design for which it is generally not possible to obtain the statistical properties of the estimated conversion factors. The paper is concluded with suggestions on how to optimize survey design. The method was used to estimate conversion factors for Atlantic cod (*Gadus morhua*) from Danish gear calibration experiments in the Baltic Sea.

Résumé : Les inventaires au chalut fournissent des données importantes pour l'évaluation des fluctuations temporelles de l'abondance relative des stocks. Lorsqu'on change les engins de pêche ou les navires qui servent à l'inventaire, il est important de comparer l'efficacité et la sélectivité des anciens et des nouveaux engins ou navires. Nous avons mis au point une méthode pour estimer les facteurs de conversion nécessaires, basée sur un plan d'inventaire dans lequel des traits de chalut appariés sont réalisés sur une même ligne de pêche. La méthode tient compte de façon explicite des changements de densité des poissons qui résultent des perturbations causées par le chalutage. Nous avons obtenu analytiquement un modèle linéaire généralisé des récoltes appariées et nous avons estimé les paramètres de conversion des engins et les paramètres de perturbation, de même que leur précision, à l'aide de logiciels communs. De plus, des études de simulation montrent que les facteurs de conversion estimés sont pratiquement sans erreur. À cause de l'indépendance de la répartition spatiale des poissons, la nouvelle méthode est préférable aux plans habituels de récoltes appariées dans lesquels il n'est généralement pas possible d'obtenir les propriétés statistiques des facteurs de conversion estimés. Nous concluons par des suggestions pour optimiser le plan d'inventaire. Nous avons utilisé la méthode pour estimer les facteurs de conversion pour des données sur la morue franche (*Gadus morhua*) provenant d'expériences danoises de caractérisation d'engins de pêche faites dans la Baltique.

[Traduit par la Rédaction]

Introduction

Time series of catch rates in trawl surveys are important sources of information for tracking the development in fish stock abundance and are often used to calibrate analytical stock assessments. Sometimes it is necessary to introduce new survey vessels or to change the fishing gear (e.g., ICES 1992; Nielsen et al. 2001), which destroys the continuity of the survey time series data. In these cases, it is necessary to compare the efficiency and the selectivity of the old and new gears (or vessels) and to estimate conversion factors that allow the catches taken in the historical period to be expressed in units compatible with the new standard.

When reviewing the literature, Pelletier (1998) noted that the experimental designs used for calibrating trawl survey gears may broadly be classified into two groups: (*i*) paired hauls methods where stations close in time and space are selected on the tacit assumption that fish density is the same (e.g., Sissenwine and Bowman 1978; Wilderbuer et al. 1998) or (*ii*) area-based methods where the fish density and size structure are assumed to be homogeneous within particular strata (e.g., Ehrich 1991). Despite the difference in survey design, the estimation of conversion factors is often based on classical statistical methods, i.e., lognormally distributed catches combined with linear models accounting for gear and location and haul effects (Robson 1966; Sissenwine and Bowman 1978; Wilderbuer et al. 1998). Other estimates are based on the relationship between catch per unit effort (CPUE) of two gears (Wilderbuer et al. 1998). Kappenman (1992) developed an estimator based on the assumption that

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the distribution of paired CPUEs of the two gears considered are identical except for the scale parameters. Warren (1997) applied a log-linear model based on the CPUE ratio. For a paired trawl design where two vessels are trawling at the same time with a distance ranging from 0.25 to 1 nautical mile, Pelletier (1998) applied a quasi-likelihood method assuming that the mean values of the abundance associated with the paired hauls are identical.

In the present paper, we consider the special case of the paired hauls design where the successive hauls are taken in the same track line within a short time range. This design has previously been used for trawl comparison (Wilderbuer et al. 1998; ICES 2002) and for comparing different coastal gears (Methven and Schneider 1998). This design is preferred because it reduces the spatial variability of fish density to a minimum and thus justifies the assumption that the fish density is the same for each of the two paired hauls. However, fishing in immediate succession in the same track creates a new problem, as the first haul may affect the fish density available for the second haul by removing fish or by inducing changes in behaviour that alters fish distribution. This effect, which is termed the disturbance effect, therefore needs to be estimated and accounted for.

The purpose of the present work is to develop a method for gear calibrations that is independent of the fish density, which is treated as nuisance parameter, and includes estimates of the conversion factor and the disturbance effect of both the new and the old gear. The method is expressed as a generalized linear model (McCullagh and Nelder 1989) that allows the precision of the parameters to be estimated and hypotheses to be tested using standard software. The method is used to analyse Danish calibration experiments carried out as part of the Baltic International Trawl Survey (Nielsen et al. 2001; ICES 2002).

Methods

Formulation of the statistical model

The statistical model is based on a survey design where two hauls are taken successively in the same track line where it is acknowledged that the first haul affects the fish density available to the second haul. The disturbance caused by the first haul is assumed to be dependent on the gear actually used but being independent of fish density and habitat. The disturbance effect is the net effect of removing the fish caught and affecting the fish behaviour, which includes both attraction of fish to and repulsion of fish from the track line. Theoretically, the disturbance effect can be both positive and negative dependent on whether fish are mainly attracted or repulsed.

When comparing two gears, a total of four possible types of experiments can be carried out. They are classified as follows: type 0, the experiment for which the old gear is applied twice; type 1, a haul of the old gear is followed by a haul of the new gear; type 2, a haul of the new gear is followed by a haul of the old gear; type 3, the new gear is applied twice.

Let $U^{\text{seq}}_{\text{type},l,s}$ denote the CPUE for a given experiment type, temporal sequence of the hauls seq, length group l, and station s, where seq = 1, 2 and type = 0, 1, 2, 3. It is assumed that all CPUEs are Poisson distributed with mean equal to gear catchability multiplied by the fish density. For the second haul, fish density is assumed to be affected by the disturbance effect. Finally, it is assumed that the CPUEs for the first and second haul given the fish density are independent variables. The model is summarized in eqs. 1–4:

	First haul	Second haul
(1) Type 0	$E(U^1_{0,l,s}) = q_{\text{old},l}D_{0,l,s}$	$E(U^2_{0,l,s}) = q_{\text{old},l}\alpha_l D_{0,l,s}$
(2) Type 1	$E(U^1_{1,l,s}) = q_{\text{old},l}D_{1,l,s}$	$E(U^2_{1,l,s}) = q_{\text{new},l}\alpha_l D_{1,l,s}$
(3) Type 2	$E(U^1_{2,l,s}) = q_{\text{new},l}D_{2,l,s}$	$E(U^2_{2,l,s}) = q_{\text{old},l}\beta_l D_{2,l,s}$
(4) Type 3	$E(U^1_{3,l,s}) = q_{\text{new},l}D_{3,l,s}$	$E(U^2_{3,l,s}) = q_{\text{new},l}\beta_l D_{3,l,s}$

where E denotes the expected value, $q_{\text{old},l}$ and $q_{\text{new},l}$ are the catchabilities of the two gears, and D is the fish density at station s prior to the first haul. For the second haul, fish density is assumed to be affected by the disturbance caused by the first haul where α_l designates the disturbance effect of the old gear and β_l the disturbance effect of the new gear. The parameters α_l and β_l may be less or greater than 1, as no assumption is made on the net effect. As the catchabilities as well as the disturbance effects may differ by length, the equations are supplied with and index l reflecting different length classes.

The objective of the survey gear calibration is to estimate the conversion factors, that is, the ratios between the catchabilities, $\gamma_l = q_{\text{new},l}/q_{\text{old},l}$. It is immediately observed that the conversion factor cannot be estimated without estimating at least one of the disturbance parameters α_l and β_l.

Now let $U_{\text{type},l,s} = U^1_{\text{type},l,s} + U^2_{\text{type},l,s}$ denote the sum of CPUEs by type, length group, and station. As the CPUEs for given fish densities are assumed to be independent Poisson distributed variables, it is well known that the conditional distributions of $U^2_{0,l,s} | U_{0,l,s}$, $U^2_{1,l,s} | U_{1,l,s}$, $U^1_{2,l,s} | U_{2,l,s}$, and $U^1_{3,l,s} | U_{3,l,s}$ are binomially distributed $B(U_{\text{type},l,s}, p_{\text{type},l})$ for each type, length group, and station and where

$$(5)\qquad p_{0,l} = \frac{E(U^2_{0,l,s})}{E(U^1_{0,l,s}) + E(U^2_{0,l,s})} = \frac{q_{\text{old},l}\alpha_l D_{0,l,s}}{q_{\text{old},l}D_{0,l,s} + q_{\text{old},l}\alpha_l D_{0,l,s}} = \frac{1}{1 + 1/\alpha_l}$$

$$(6)\qquad P_{1,l} = \frac{E(U^2_{1,l,s})}{E(U^1_{1,l,s}) + E(U^2_{1,l,s})} = \frac{\gamma_l}{\gamma_l + 1/\alpha_l}$$

$$(7)\qquad P_{2,l} = \frac{E(U^1_{2,l,s})}{E(U^1_{2,l,s}) + E(U^2_{2,l,s})} = \frac{\gamma_l}{\gamma_l + \beta_l}$$

$$(8)\qquad P_{3,l} = \frac{E(U^1_{3,l,s})}{E(U^1_{3,l,s}) + E(U^2_{3,l,s})} = \frac{1}{1 + \beta_l}$$

Equations 5–8 show that estimation of the absolute values of the catchabilities is not necessary (or possible).

The logits, $r_{\text{type},l}$, of the four probabilities are

$$r_{0,l} = \text{logit}(p_{0,l}) = \ln\left(\frac{p_{0,l}}{1 - p_{0,l}}\right) = \ln(\alpha_l) = \theta_l$$

$$r_{1,l} = \text{logit}(p_{1,l}) = \ln(\gamma_l) + \ln(\alpha_l) = \pi_l + \theta_l$$

$$r_{2,l} = \text{logit}(p_{2,l}) = \ln(\gamma_l) - \ln(\beta_l) = \pi_l - \tau_l$$

$$r_{3,l} = \text{logit}(p_{3,l}) = -\ln(\beta_l) = -\tau_l$$

where

$$\pi_l = \ln(\gamma_l) = \ln\left(\frac{q_{\text{new},l}}{q_{\text{old},l}}\right), \; \theta_l = \ln(\alpha_l), \text{ and } \tau_l = \ln(\beta_l).$$

The four logits, $r_{\text{type},l}$, type = 0–3, can be combined into one linear equation:

$$(9) \qquad r_{\text{type},l} = \pi_l x_{\text{type}} + \theta_l y_{\text{type}} + \tau_l z_{\text{type}}$$

where

$$x_{\text{type}} = \begin{cases} 0 & \text{if type} = 0 \\ 1 & \text{if type} = 1 \\ 1 & \text{if type} = 2 \\ 0 & \text{if type} = 3 \end{cases}$$

$$y_{\text{type}} = \begin{cases} 1 & \text{if type} = 0 \\ 1 & \text{if type} = 1 \\ 0 & \text{if type} = 2 \\ 0 & \text{if type} = 3 \end{cases}$$

$$z_{\text{type}} = \begin{cases} 0 & \text{if type} = 0 \\ 0 & \text{if type} = 1 \\ -1 & \text{if type} = 2 \\ -1 & \text{if type} = 3 \end{cases}$$

As the conditional distributions derived are binomial distributions and the logits (the canonical link function) are linear functions of the parameters, π_l, θ_l, and τ_l, the theory of generalized linear models (McCullagh and Nelder 1989) was applied to analyse the paired CPUE data. The model may be regarded as multiple linear regression with heterogeneous slopes and with x, y, and z as known covariates.

If the assumption of binomial distributed variables does not hold, an overdispersion parameter is estimated (McCullagh and Nelder 1989). Testing whether the conversions factors γ_l and the disturbance effects α_l and β_l differ across length classes is facilitated by the standard reformulation of eq. 9 to

$$r_{t,l} = \pi x_t + \pi_l x_t + \theta y_t + \theta_l y_t + \tau z_t + \tau_l z_t$$

and by testing the hypotheses $\pi_l = \theta_l = \tau_l = 0$.

The GENMOD SAS procedure (SAS Institute Inc. 1996) was used to estimate parameters and testing hypotheses. The Pscale option and Wald's statistics were used as a test to include overdispersion. Type3 contrast instead of Type1 analysis (SAS notation) was carried out because this is recommended in connection with Wald's statistics. This means that only the result of the first hypothesis tested can be used. When testing a new hypothesis, a new run must be carried out with a new model specifying the factors in the correct order.

Simulation study

Two main types of studies were carried out with the following purposes. The first was to investigate the bias of the estimated conversion factors using the new method based on the survey design where paired hauls are taken in the same trawl track line and, further, to compare the properties of the estimator derived with an alternative estimator. In this study, it was assumed for each station that the catches were based on fixed fish densities implying that only the catches generated varied in the simulations. All simulations were carried out for each length group separately and were based on the observed CPUEs and estimated values of both conversion factors and disturbance effects to reflect the conditions observed in the field experiments.

The second was to compare the new method with a paired hauls design, where the distance between nearby hauls is chosen to be as small as possible but without any kind of disturbance. The performance of the traditional paired hauls design heavily depends on the assumptions on spatial fish distribution. For the simulations comparing the two methods, a spatial distribution pattern has been chosen that is favourable for the traditional paired hauls design. The basic assumption is that the two fish densities at each of the paired nearby stations were drawn from the same statistical distribution. It was further assumed that the variance was the same for all stations. The variance measures the spatial inhomogeneity of the resource. One would expect that the paired hauls method is superior to the new method when the variance is small and vice versa. Both simulation procedures are described in Appendix B.

Optimal survey design

The field experiments included experiments of types 1–3 and enabled estimation of the conversion factor and the disturbance factor associated with both gears. However, as, shown in Appendix A, if the full model eq. 9 is used, the estimator of the conversion factor only depends on data from experiments of types 2 and 3. The conversion factor could alternatively be estimated using experiments of types 0 and 1. The researcher should thus attempt to evaluate which of these two designs will lead to the most precise conversion factor estimate for a given research effort.

As shown in Appendix A, the conversion factor for a given length group applying data of types 2 and 3, $\hat{\gamma}_{2+3}$ is estimated by

$$\hat{\gamma}_{2+3} = \hat{\beta}\frac{1 - \hat{p}_2}{\hat{p}_2}$$

where

$$\hat{\beta} = \frac{1 - \hat{p}_3}{\hat{p}_3}$$

where $\hat{p}_2$ and $\hat{p}_3$ are defined by eq. A4 in Appendix A.

The variance of the estimated conversion factor can be approximated by

Table 1. Ground gear rigging and Scanmar trawl size measurements from the Danish calibration experiments in the Baltic Sea.

		Gear dimensions	
Gear	Ground gear rigging	Trawl height (m)	Door spread (m)
TV3	Rubber snake with inserted 15-cm rubber discs	6.0–8.0	60–85
Granton	Iron bobbins, diameter 40 cm	3.0–4.5	60–75

$$(10)\quad V(\hat{\gamma}_{2+3}) \approx \beta^2 \mathrm{VAR}\left(\frac{1-\hat{p}_2}{\hat{p}_2}\right) + \left(\frac{1-\hat{p}_2}{\hat{p}_2}\right)^2 \mathrm{VAR}(\hat{\beta}) = \left(\frac{\beta}{\gamma}\right)^3 \left(\frac{(\gamma+\beta)^2}{n_2} + \frac{(1+\beta)^2\gamma}{n_3}\right)$$

where n_2 and n_3 are the total number fish caught for both gears at all stations for type 2 and type 3 experiments, respectively. If n_{2+3} $(= n_2 + n_3)$ is total number fish caught in the type 2 + type 3 experiments, the optimal distribution on the two types is (Raj 1968)

$$\frac{n_2^{\text{optimal}}}{n_{2+3}} = \frac{\gamma+\beta}{\gamma+\beta+(1+\beta)\sqrt{\gamma}}$$

$$\frac{n_3^{\text{optimal}}}{n_{2+3}} = 1 - \frac{n_2^{\text{optimal}}}{n_{2+3}}$$

Instead of a formulation based on number of fish caught, we prefer an equation that is based on number of hauls. The total mean number of fish caught at types 2 and 3 are proportional to $\gamma + \beta$ and $\gamma(1 + \beta)$, respectively. Using these values as relative weights, the optimal allocation of the number of hauls by the two types, m_2^{optimal} and m_3^{optimal}, becomes

$$(11)\quad \frac{m_2^{\text{optimal}}}{m_{2+3}} = \frac{\sqrt{\gamma}}{1+\sqrt{\gamma}}, \quad \frac{m_3^{\text{optimal}}}{m_{2+3}} = \frac{1}{1+\sqrt{\gamma}}, \text{ and}$$

$$m_{2+3} = m_2^{\text{optimal}} + m_3^{\text{optimal}}$$

The optimal solution only depends on the conversion factor and not on the disturbance. If $\gamma > 1$, i.e., the new gear is more efficient than the old gear, a larger proportion should be taken of the type 2 experiments and vice versa. For $\gamma = 1$, the optimal split is 0.5 for each of the type of experiments.

The minimum variance for type 2 + type 3 experiments, which corresponds to the optimal solution, is found by insertion of n_1^{optimal} and n_2^{optimal} into eq. 10:

$$V^{\min} = (\hat{\gamma}_{2+3}) \approx \frac{1}{n_{2+3}} \frac{\gamma}{\beta} (\beta + \gamma + (1+\beta)\sqrt{\gamma})^2$$

Similarly applying type 0 + type 1 experiments, the optimal minimum variance is

$$V^{\min}(\hat{\gamma}_{0+1}) \approx \frac{1}{n_{0+1}} \gamma\alpha \left(\frac{1}{\alpha} + \gamma + \left(1 + \frac{1}{\alpha}\right)\sqrt{\gamma}\right)^2$$

The latter two equations are based on total the number of fish caught in both types 2 + 3 and 0 + 1, n_{2+3} and n_{0+1}, respectively. Again, we prefer to compare variances that are based on the number of hauls, as the total mean numbers of fish caught are proportional to $2 + \alpha(1 + \gamma)$ and $2\gamma + \beta(1 + \gamma)$ for types 0 + 1 and 2 + 3, respectively. The minimum variances can be approximated by

$$(12)\quad V^{\min}(\hat{\gamma}_{2+3}) = \frac{1}{m_{2+3}} \frac{1}{(2\gamma + \beta(1+\gamma))} \frac{\gamma}{\beta} (\beta + \gamma + (1+\beta)\sqrt{\gamma})^2$$

$$(13)\quad V^{\min}(\hat{\gamma}_{0+1}) = \frac{1}{m_{0+1}} \frac{1}{(2 + \alpha(1+\gamma))} \times \gamma\alpha \left(\frac{1}{\alpha} + \gamma + \left(1 + \frac{1}{\alpha}\right)\sqrt{\gamma}\right)^2$$

where m_{2+3} and m_{0+1} designate the total number of hauls.

Comparative fishing trials comparing survey gears

The survey gear calibration data were collected within a European Union Study Project (Nielsen et al. 2001). The objective of this project was to standardize the national abundance surveys in the Baltic Sea by introducing a common survey gear and to develop an internationally coordinated survey design. Here, the analysis is restricted to the Danish fishing experiments.

The Danish research vessel R/V *Dana* carried out the comparative fishing operations during the standard Baltic Atlantic cod (*Gadus morhua*) surveys in the first and fourth quarters during the period 1999–2002. The calibration stations were selected based on catch rates information available from the ongoing survey in an attempt to avoid comparisons in areas with low cod density. The gears compared were the Granton trawl traditionally used on the Danish surveys (ICES 1997) and the TV3 trawl, which is the new international standard (Nielsen et al. 2001; ICES 2002). The two gears differ by design and size and are equipped with different ground gears (Table 1). The fishery was only performed during daytime (light hours).

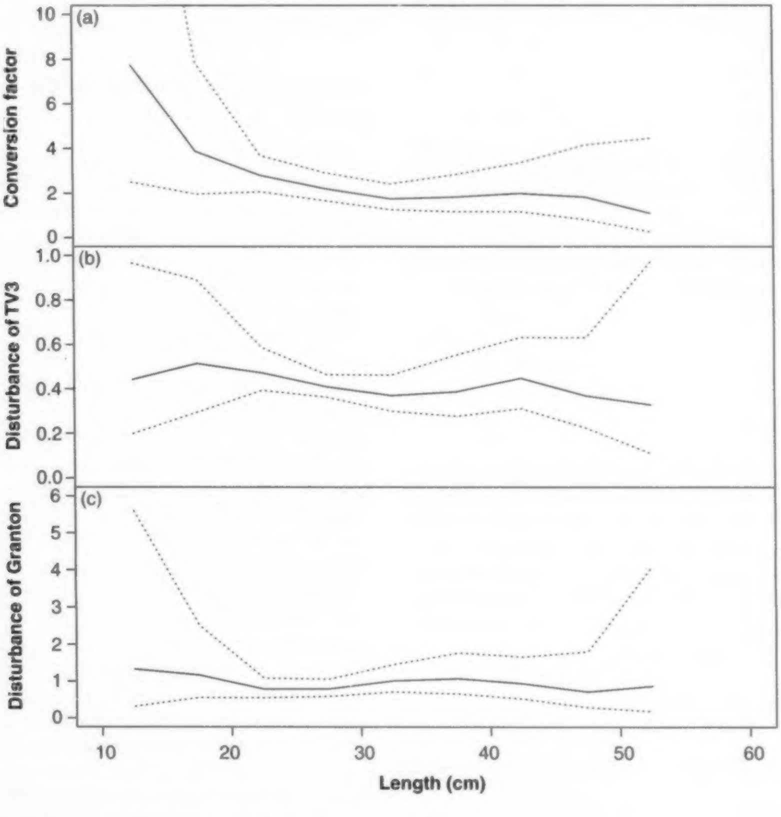
The paired hauls were made by conducting the vessel's second haul on the global positioning system track position line available from the first haul and in the same direction. The second haul was made immediately after the first, i.e., commenced within 0.75–1 h after the retrieval of the first haul. The duration of each haul was 30 min (±2 min) carried out with the same trawling speed of about 3 kn. The field experiments were restricted to the types 1–3, i.e., omitting trials replicating tows with the old gear (Table 2).

Before analysis, the catch data were standardized to 30-min haul duration and were grouped into 5-cm length groups (rounded numbers because the binomial distribution model applied demands integer values). A few haul pairs were ex-

Table 2. Number of Baltic Sea stations with paired hauls and average number of fish caught per station by type of experiment, gear, and length group.

	Average number of fish caught by type of experiment					
	Type 1 (old gear – new gear)		Type 2 (new gear – old gear)		Type 3 (new gear – new gear)	
Length group (midpoints) (cm)	Granton haul 1	TV3 haul 2	TV3 haul 1	Granton haul 2	TV3 haul 1	TV3 haul 2
12.5	0.9	9.8	15.7	0.9	12.2	5.3
17.5	8.9	39.5	30.0	4.1	15.8	8.1
22.5	41.3	90.6	55.7	9.2	161.9	76.9
27.5	90.4	157.3	47.8	8.9	299.4	122.8
32.5	38.9	68.6	51.5	11.0	137.1	50.6
37.5	15.5	30.5	41.3	8.6	47.6	18.1
42.5	10.6	20.0	19.5	4.5	43.0	19.6
47.5	6.7	8.6	7.5	1.5	23.8	8.6
52.5	2.8	2.7	3.5	1.0	6.6	2.1
No. of stations	24	28	8			

Fig. 1. Estimates (solid line) and 95% confidence limits (broken lines) by length of conversion factors for (*a*) Granton converted to TV3, (*b*) disturbance effects of the TV3 trawl, and (*c*) disturbance effects of the Granton trawl.



cluded from the analysis because that total number of fish caught per haul was especially low, less than 20 individuals. Cod less than 10 cm were not recruited fully to any of the gears and were therefore omitted from the analysis. Similarly, the largest cod, i.e., cod greater than 55 cm in length, were excluded from the analyses because of a low number of fish caught.

Results

The model eq. 9 was applied to the Danish comparative trawl experiment data. The estimated conversion factors show a clear decline with fish length from values above 9 for the smallest cod sizes to values between 1 and 2 for cod greater than 25 cm. (Fig. 1*a*). In contrast, for the disturbance

Table 3. Test results of hypotheses H_1–H_4 using Type3 analysis (SAS notation).

Test	df	χ^2 (Type3)	$P > \chi^2$
H_1: No overdispersion	490	1749	<0.0001
H_2: $\gamma_l = \gamma$	8	14.22	<0.0001
H_3: $\alpha_l = \alpha$	8	3.76	0.4391
H_4: $\beta_l = \beta$	8	4.77	0.7822

Table 4. Conversion factors, γ_l, and lower and upper confidence limits (CL) by length for the conversion of Granton catches to TV3 units.

Length (cm)	Conversion factor	Lower 95% CL	Upper 95% CL
12.5	9.19	4.93	17.12
17.5	4.17	3.21	5.43
22.5	2.46	2.06	2.95
>22.5	1.96	1.69	2.27

Table 5. Estimated disturbance parameters of the Granton trawl (α) and the TV3 trawl (β) and their lower and upper confidence limits (CL).

	Disturbance	Lower 95% CL	Upper 95% CL
α	0.90	0.77	1.05
β	0.42	0.38	0.46

factors (α_l and β_l), no clear systematic trends with cod size were observed (Figs. 1*b* and 1*c*).

Four hypotheses were tested:

$$H_1\text{: No overdispersion}$$

$$H_2\text{: } \gamma_l = \gamma \Leftrightarrow \pi_l = \pi$$

$$H_3\text{: } \alpha_l = \alpha \Leftrightarrow \theta_l = \theta$$

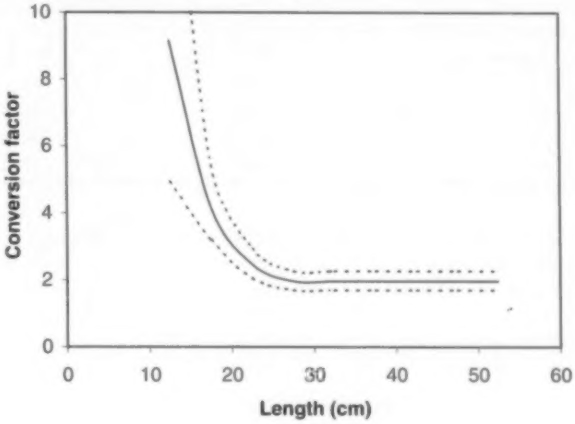
$$H_4\text{: } \beta_l = \beta \Leftrightarrow \tau_l = \tau$$

The dispersion was estimated at 1.89, which is significantly larger than 1, indicating that the conditional distribution considered is overdispersed compared with the binomial distribution assumed (Table 3). The tests of the hypotheses H_3 and H_4 were accepted and showed in both cases a clear independence of fish length (Table 3). However, the hypothesis H_2 that the conversion factors were the same for all length groups was rejected. The tests applied requires that the expected value of $U^{\text{seq}}_{\text{type},l,s}$ is larger than 5. This is the case for all length groups except for the largest fish larger than 50 cm. For the latter group, the results of the χ^2 test should be treated with caution.

To model the relationship between the conversion factors and length, the model eq. 9 was modified by expressing π_l as continuous variables in the form of a third-degree polynomial and by assuming the that disturbance factors are independent of fish size:

$$r_{\text{type},l} = (al^3 + bl^2 + cl + d)x_{\text{type}} + \theta y_{\text{type}} + \tau z_{\text{type}}$$

Fig. 2. Estimated conversion factors (solid line) and 95% confidence limits (broken lines) by length for Granton converted to TV3 using the final model (eq. 10).



Successive testing within this model showed that all coefficients of the polynomial of third degree were significant different from zero, which indicates that the conversion factor depends on the length. The estimated conversion factors, however, for fish larger than 25 cm were found to be very similar (Fig. 1*a*), and when removing fish less than 25 cm from the analysis, the hypothesis $a = b = c = 0$ was accepted, indicating that the conversion factor is the same for fish larger than 25 cm. For this reason, fish greater than 25 cm were aggregated into a single plus group. This led to the final model where the conversion factors differ for the four length classes, whereas both disturbance factors are independent of length:

$$r_{\text{type},l} = \pi_l x_{\text{type}} + \theta y_{\text{type}} + \tau z_{\text{type}}$$

for the length groups l = 12.5, 17.5, 22.5, and >25 cm.

In the final model, the conversion factor decreases from a value of approximately 9 for the smallest length class to a plateau of approximately 2 for fish greater than 25 cm (Fig. 2; Table 4). For none of the length classes does the confidence interval include the value of 1, implying that for all sizes of cod, the new trawl is significantly more efficient than the old trawl. The confidence limits are much wider for small fish of length 10–15 cm because of a low number of fish caught. Both disturbance parameters are independent of length. For the TV3 trawl, the disturbance factor is estimated at 0.42 and is found to be significantly different from 1, implying that fishing with this gear lowers the availability of cod in the short run. The disturbance factor for the Granton trawl is estimated at 0.90 and is not found to be statistically different from 1 (Table 5).

Simulation study

Simulation study 1, which assesses the relative bias and mean square error (MSE) of the estimators of the conversion factors associated with the new method (paired hauls taken in the same track lines), was carried out for two alternative estimators (the ML estimator $\hat{\gamma}_1$ used in the model and $\hat{\gamma}_2$ being the geometric mean over stations; see Appendix B). The bias and MSE were generally found to be very low ex-

Table 6. Relative bias and MSE of $\hat{\gamma}_1$ and $\hat{\gamma}_2$ by length group obtained from simulation study 1 (Appendix B).

	Relative bias		MSE	
Length group (midpoints) (cm)	$(\hat{\gamma}_1/\gamma-1)$	$(\hat{\gamma}_2/\gamma-1)$	$\hat{\gamma}_1$	$\hat{\gamma}_2$
12.5	0.16	–0.39	36.6	22.0
17.5	0.03	–0.18	1.5	2.11
22.5	0.01	–0.003	0.08	0.16
>22.5	0.003	–0.07	3×10^{-5}	0.02

Fig. 3. MSE of $\hat{\gamma}_1$ (new method: broken line) and $\hat{\gamma}_4$ (paired hauls: solid line) versus mean absolute value of relative deviation between fish densities at two parallel track lines, $E_D(\sigma)$, for the Atlantic cod size group 20–25 cm. At the intersection $E_D(\sigma_0)$, the two methods provide results of equal quality.

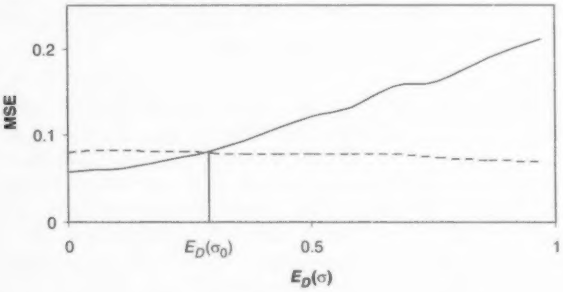


Table 7. Comparison of the new method ($\hat{\gamma}_1$) with the paired hauls method ($\hat{\gamma}_4$) obtained from simulation study 2.

		Relative bias	
Length group (midpoints) (cm)	$E_D(\sigma_0) = E(\lvert D_1/D_2 - 1\rvert)$	$(\hat{\gamma}_1/\gamma-1)$	$(\hat{\gamma}_4/\gamma-1)$
12.5	4.50	0.10	–0.43
17.5	1.30	0.03	–0.17
22.5	0.30	0.01	0.03
>22.5	0.31	0.003	–0.02

Note: The mean absolute value $E_D(\sigma_0)$ of relative deviation between fish densities at two nearby hauls, D_1 and D_2, for which the MSE of the new method equals that of the paired hauls estimator $\hat{\gamma}_4$ and relative bias of the two estimators (see Appendix B).

Table 8. Ratio of the minimum variances of the conversion factors estimated by either type 2 + type 3 or type 0 + type 1 experiments when assuming equal effort ($m_{0+1} = m_{2+3}$).

Length group (cm)	$\frac{V^{\min}(\hat{\gamma}_{2+3})}{V^{\min}(\hat{\gamma}_{0+1})}$*
12.5	0.91
17.5	1.02
22.5	1.17
>22.5	1.26

*Equation 12/eq. 13.

cept for the smallest fish size class where catches were low (Table 6). The MSE of $\hat{\gamma}_1$ was found to be less than the MSE of $\hat{\gamma}_2$ for all length groups except for the 12.5 cm group, i.e., for more than 99% of the fish caught with the old Granton trawl. For the 12.5 cm group, however, the bias of $\hat{\gamma}_2$ is more than double of that of $\hat{\gamma}_1$. Overall, we therefore conclude that the estimator $\hat{\gamma}_1$ is superior to $\hat{\gamma}_2$. As a consequence, estimator $\hat{\gamma}_1$ is used for the new method in simulation study 2.

Simulation study 2 was initiated by assessing the most efficient estimator of the conversion factor associated with the traditional paired hauls design. It was here found that the estimator $\hat{\gamma}_4$ was superior and $\hat{\gamma}_4$ was therefore used for that method (Appendix B).

The relationship between spatial inhomogeneity ($E_D(\sigma)$), which measures the relative difference between the density of the two paired hauls stations, and MSE of the two methods shows that the MSE of the new method ($\hat{\gamma}_1$) is approximately constant (i.e., independent of the fish distribution), while the MSE of the traditional paired hauls method ($\hat{\gamma}_4$) is an increasing function of the spatial inhomogeneity (Fig. 3 shows the results for the 20–25 cm length group). As the MSE of the paired hauls design is less than the MSE of the new method at complete spatial homogeneity, the two curves intersect at a point denoted $E_D(\sigma_0)$. When $E_D(\sigma) > E_D(\sigma_0)$, the new method is preferable and vice versa. The interception points ($E_D(\sigma_0)$) differ between the size classes of cod because of the difference in observed catches (Table 7).

The $E_D(\sigma_0)$ is about 30% for fish larger than 20 cm that accounted for 95% of the fish caught with the old and inefficient Granton trawl. For small fish, which were poorly represented in the Granton trawl catches, the interception point is much higher, 450% and 130% for length groups 12.5 and 17.5, respectively. However, the bias in the conversion factors associated with the traditional paired hauls design is substantial for the small size classes and significantly higher than found with the new method (Table 7).

Optimal survey design

Based on the conversion and disturbance factors estimated and assuming equal effort (i.e., $m_{0+1} = m_{2+3}$), the relationship between the variances determined by eqs. 12 and 13 has been calculated (Table 8). The strategy based on experiments of types 2 + 3 should be preferred if the relationship is less than 1. The table shows that that the differences between the two strategies are small. Strictly following the results, strategy 2 + 3 should be preferred for fish less than 15 cm, whereas strategy 0 + 1 should be preferred for the larger fish.

The calculation of the optimal design is based on the assumption of the binomial distribution. However, the analyses showed that the observations were overdispersed compared with the binomial distribution. The calculations provided should therefore be regarded as an approximation to the optimal design.

Discussion

There are a number of approaches available for estimating relative fishing powers between different vessels or gears. The approaches have different merits and faults and the selection of the most appropriate method will depend on the scientific scope, the resources available, and the peculiarities of the biological system studied. A major issue is the distinction between designated experiments and the use of existing data as available from scientific surveys or commercial catch rates (e.g., Robson 1966; Gavaris 1990; Sparholt and Tomkiewicz 2000). For the designated experiments, the paired hauls experimental design is an appealing approach when stations close in time and space are more similar than stations widely spaced. Wilderbuer et al. (1998) analysed survey data covering a wide depth range and found for that case that fishing power was more precisely estimated by the methods that explicitly used the paired hauls information available. However, a number of papers have demonstrated considerable spatial variability in catch rates between nearby stations (e.g., Ehrich 1991; Kvist et al. 2001), implying that nearby hauls do not cover the same population, which means that the benefits of paired nearby stations vanish. Further, in practice, information on the spatial distribution of the resource populations is rarely available.

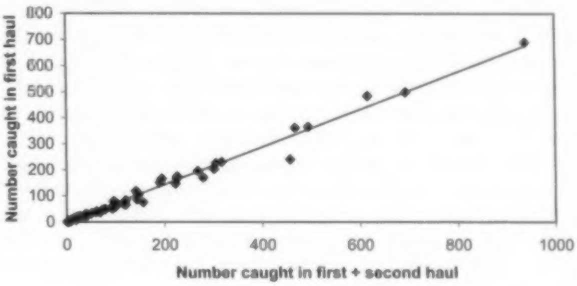
The key quality of the method developed here is that the gear comparisons are conducted at the same sites, implying that it is independent of the finer scale spatial distribution of the resources. It is assumed that density and size structure of the resource are retained at the station site within the short duration between successive tows except for the systematic and estimable disturbance effects caused by the fishing operations. The method has the additional merits in that it uses standard statistical techniques that allow maximum likelihood estimation of parameters, calculation of confidence limits of the estimates, and testing of hypotheses. The simulation studies additionally showed that the estimated conversion factors were practically unbiased for size groups that accounted for more than 99% of the fish caught.

For the traditional paired hauls comparisons, it is not possible to assess bias and uncertainties of the conversion factors because they depend on the fine-scale spatial distribution of the resources, which is not known.

For a special case of spatial distribution for which strict assumptions have to be fulfilled, we presume that it is possible to obtain the statistical properties of conversion factor estimates from a paired hauls design experiment by, e.g., Markov Chain Monte Carlo or Bayesian techniques. The assumptions are that the densities from nearby stations originated from the same statistical distribution and that the variance of the densities were equal across all stations fished. Simulations carried out for this case showed that increasing spatial inhomogeneity led to poorer performance of the traditional paired hauls method, whereas the new method was insensitive to the homogeneity differences. For the present Baltic Sea case, the simulations indicated that for the larger cod (accounting for 95% of the catch), the new method was preferable when the mean density difference between two paired stations exceeded 30%.

The new TV3 trawl was significantly more efficient than the Granton trawl hitherto used, especially for cod less than 20 cm. This is in line with the a priori expectations, as the new trawl was larger both vertically and horizontally and used a rubber snake ground gear instead of the bobbin arrangement used earlier. This new ground rope arrangement was suggested as the survey standard (ICES 1997), acknowledging the body of research that has documented the scope of enhancing the catchability of small cod owing to its ability to follow bottom (Engås and Godø 1989; Walsh 1989; Ehrich 1991).

Fig. 4. Number caught in the first haul versus number caught in the first haul plus the second haul by station and length group for type 3 experiments. The line shows the expected value based on the estimated β.



The analyses showed that the disturbance effect of the TV3 trawl was quite significant, being estimated at about 0.4, implying that the fish density available for the subsequent haul was reduced by 60%. Although the disturbance effect is not of primary interest when calibrating gear efficiency, its inclusion in the model crucially affects the estimated size of the conversion factors. This may be illustrated by contrasting the present approach with that used by Wilderbuer et al. (1998) who ignored the disturbance factors but attempted to balance out the sequence effect by splitting their survey effort equally between the type 2 and type 3 experiments. When using this approach, the conversion factor can be expressed as $\gamma = 0.5(\alpha + 1/\beta)(q_{\text{new}}/q_{\text{old}})$. Inserting the disturbance information available from the present study shows that by ignoring disturbance effects, the conversion factor would be overestimated by 64%.

The disturbance effect introduced here is conceptually related to the depletion effects known from population size estimation from consecutive fishing on a closed population (i.e., Leslie and deLury method used in freshwater fishing; cf. Ricker 1975). However, the disturbance effect designates a net effect that includes both the removal of fish caught by the first fishing operation and induced behavioural effects that influence migration of fish in the neighbourhood of the trawl track line. Because of the nonclosed nature of a trawl operation, it is impossible to distinguish between these two effects. The estimated disturbance parameters are nevertheless related to the size of the catch as reflected by the catchabilities. The disturbance effect of the relative inefficient Granton trawl was thus estimated at 0.90, which was not significantly different from 1.0, whereas the disturbance effect of the more efficient TV3 trawl was estimated at 0.4 and was clearly found to be significantly different from 1.0.

For the approach taken here, it is assumed that both disturbance effects and conversion factors depend only on the size of the fish but not on any station or habitat characteristics such as depth, fish abundance, or bottom type. Although the present experiments were not designed to illuminate the importance of station differences, a simple graphical evaluation of such possible effects was made by plotting CPUE for the first haul against the sum of CPUEs of the first and second hauls for the type 3 experiments (Fig. 4). According to the model, the observations from each station and length class should lie around a line through the origin with a slope equal to $1/(1 + \beta_l) = 0.7$ as $\beta_l = \beta = 0.42$. No stations were found to be obvious outliers and no trends in residuals pattern by density were observed, indicating that the fish density did not influence the disturbance effect.

With the present model formulation, the estimates and the uncertainty of the parameters depend only on the number of fish caught and not on the number or type of stations covered, implying that if it is possible to catch a high number of fish at a few stations, this should be sufficient to obtain suitable conversion factors. However, it cannot be precluded that systematic station differences may imply that catchability and disturbance differ across habitats, and if such factors are identified as important, the experimental design and the model should be changed accordingly, e.g., by including the factor in the generalized linear model (eq. 9).

The section on survey design notes that for the present model, the conversion factors can be estimated by carrying out either type 2 (new gear – old gear) and type 3 (new gear – new gear) experiments or type 0 (old gear – old gear) and type 1 (old gear – new gear) experiments.

When prior information on conversion and disturbance factors is available, which is the case when the experimental hauls are not restricted to a single survey, the precision of gear conversion estimates for the two alternatives may be evaluated, enabling the researcher to choose the optimal survey design. Within the strategy chosen, the researcher may subsequently choose the optimum allocation of hauls between the two types of experiments involved. Furthermore, for a specified level of uncertainty associated with the conversion factors estimated, the number of paired stations required can be determined.

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Appendix A

In some cases, observations of type 0 (paired replications using the old trawl) are not available because it is not obvious if they are necessary to estimate conversion factors. Therefore, if it is assumed that only observations for types 1, 2, and 3 are available, the likelihood function for a given length group l is a product of three binomial distributions:

$$L_l((C_{t,l,s}|U_{t,l,s})) = \prod_{t=1}^{3}\prod_{s}\binom{U_{t,l,s}}{C_{t,l,s}} p_{t,l}{}^{C_{t,l,s}}(1 - p_{t,l})^{U_{t,l,s}-C_{t,l,s}}$$

where $C_{1,l,s} = U^2_{1,l,s}$, $C_{t,l,s} = U^1_{t,l,s}$, and $t = 2, 3$ and $p_{t,l}$, $t = 1, 2, 3$, is defined by eqs. 6–8.

It can be shown that the maximum likelihood estimates of the parameters are

$$\text{(A1)}\quad \hat{\beta}_l = \frac{1-\hat{p}_{3,l}}{\hat{p}_{3,l}} = \frac{\sum_s U^2_{3,l,s}}{\sum_s U^1_{3,l,s}}$$

$$\text{(A2)}\quad \hat{\gamma}_l = \hat{\beta}_l \frac{\hat{p}_{2,l}}{(1-\hat{p}_{2,l})} = \hat{\beta}_l \frac{\sum_s U^1_{2,l,s}}{\sum_s U^2_{2,l,s}}$$

$$\text{(A3)}\quad \hat{\alpha}_l = \frac{1}{\hat{\gamma}_l}\frac{\hat{p}_{1,l}}{(1-\hat{p}_{1,l})} = \frac{1}{\hat{\gamma}_l}\frac{\sum_s U^1_{1,l,s}}{\sum_s U^2_{1,l,s}}$$

where

$$\text{(A4)}\quad \hat{p}_{t,l} = \frac{\sum_s C_{t,l,s}}{\sum_s U_{t,l,s}}$$

Equations A1 and A2 show that the estimated disturbance parameter $\hat{\beta}_l$ solely depends on the type 3 experiments, whereas the estimated conversion factor $\hat{\gamma}_l$ solely depends on the experiments of types 2 and 3 (and not type 1). This means that when experiments of types 1, 2, and 3 are available, the type 1 experiments do not contain information on the conversion factor but only information on the disturbance parameter α_l. However, if type 0 experiments were available, data from all four experiments would contribute to the estimation of all three parameters.

In situations where the disturbance effect of the old gear, α_l, is the same for some length groups, eqs. A1–A3 do not hold and the estimates of γ_l depend on observations from all three types of experiments.

Appendix B. Simulation studies

Study 1. Investigation of the bias of the new method and comparison with an alternative estimator

In this study, it is assumed that for each station the catch simulations are based on the same fish density such that only the generated catches vary in the simulations. All simulations were carried out for each of the length groups separately and were based on the observed 28 type 2 and 8 type 3 to match the current observations. The length groups for the large fish were aggregated as in the analyses. The simulations were based on the parameters estimated to reflect the empirical observations.

First, the fish densities for a given length group at each station were generated. The (unknown) catchability of the new gear, $q_{\text{new},l}$, was arbitrarily set to 0.5. The densities at each station during the first haul were calculated by $D_{t,l,s} = u^1_{t,l,s}/q_{\text{new},l}$, where $u^1_{t,l,s}$ is the observed CPUE and t = 2, 3, which ensures that the observed relative density distribution is applied. The catchability of the old gear was calculated by $q_{\text{old},l} = q_{\text{new},l}/\gamma_l$. The initial choice of $q_{\text{new},l}$ does not affect the analyses, as the CPUE data simulated are the same irrespective of the chosen catchability.

CPUE was assumed to follow a negative binominal (NB) distribution to reflect that the observed conditional distributions, $U^1_{t,l,s}|u_{t,l,s}$, were overdispersed compared with the binomial distribution. Accordingly, it was assumed that

$$\text{(B1)}\quad U^1_{t,l,s} \sim \text{NB}\left(q_{\text{new}} D_{t,l,s}\frac{p}{(1-p)}, p\right), t = 2, 3$$

$$\text{(B2)}\quad U^2_{t=2,l,s} \sim \text{NB}\left(\beta q_{\text{old}} D_{t=2,l,s}\frac{p}{(1-p)}, p\right)$$

$$\text{(B3)}\quad U^2_{t=3,l,s} \sim \text{NB}\left(\beta q_{\text{new}} D_{t=3,l,s}\frac{p}{(1-p)}, p\right)$$

This parameterization implies that $E(U^1_{t,l,s}) = q_{\text{new}} D_{t,l,s}$, $E(U^2_{t=2,l,s}) = \beta\, q_{\text{old}} D_{t=2,l,s}$, and $E(U^2_{t=3,l,s}) = \beta\, q_{\text{new}} D_{t=3,l,s}$, and that $\text{VAR}(U^i_{t,l,s}) = E(U^i_{t,l,s})/p$, $i = 1, 2$. According to the last equation, $1/p$ denotes the overdispersion in the NB distribution compared with the Poisson distribution. The parameter p has been chosen such that $1/p$ equals the overdispersion of $U^1_{t,l,s}|U_{t,l,s}$ compared with the binomial distribution found in the analyses (see Appendix C).

For each length group, station, and type of experiment, 10 000 set of pairs of CPUEs, $U^1_{t,l,s}(i), U^2_{t,l,s}(i))$, $i = 1,...,10\,000$, were randomly drawn from the NB distributions B1–B3. For each simulation, the conversion factor was estimated by the maximum likelihood estimator

$$\text{(B4)}\quad \hat{\gamma}_{1,l}(i) = \frac{\sum_s U^2_{3,l,s}(i)\sum_s U^1_{2,l,s}(i)}{\sum_s U^1_{3,l,s}(i)\sum_s U^2_{2,l,s}(i)}$$

using eqs. A1 and A2 in Appendix A.

An alternative estimator based on geometric averages, $\hat{\gamma}_{2,l}(i)$, was considered as well:

$$\hat{\gamma}_{2,l}(i) = \left(\frac{U^2_{3,l,1}(i)}{U^1_{3,l,1}(i)} \times \ldots \times \frac{U^2_{3,l,T}(i)}{U^1_{3,l,T}(i)}\right)^{1/T} \times \left(\frac{U^2_{2,l,1}(i)}{U^1_{2,l,1}(i)} \times \ldots \times \frac{U^2_{2,l,S}(i)}{U^1_{2,l,S}(i)}\right)^{1/S}$$

where S and T are the number of type 2 and type 3 stations, respectively.

The estimators have been compared using the relative bias $\gamma_{j,l}/\gamma_l - 1$ and the mean squared error MSE = $E(\hat{\gamma}_{j,l} - \gamma_l)^2 = \text{bias}^2 + \text{VAR}(\hat{\gamma}_{j,l})$ as measures of the goodness of the estimators, where γ_l are the assumed true values estimated. The mean of the two estimators has been estimated by $\hat{\gamma}_{j,l} = \frac{1}{10\,000}\sum_{i=1}^{10\,000}\hat{\gamma}_{j,l}(i)$, the relative bias by $\hat{\gamma}_{j,l}/\gamma_l - 1$, and MSE by

$$\widehat{\text{MSE}}_{j,l} = \frac{1}{10\,000}\sum_{i=1}^{10\,000}(\hat{\gamma}_{j,l}(i) - \gamma_l)^2 \text{ for } j = 1, 2$$

Study 2. A case-specific comparison of the new method and the paired hauls design

The assumption in this study is that the fish densities at each paired nearby station originate from the same statistical distribution. The number of paired stations was the same as in simulation study 1, namely 28 type 2 and 8 type 3 experiments, i.e., 36 in total.

Let $D_l = \{D_{t=2,l,s=1},\ldots, D_{t=2,l,s=28}, D_{t=3,l,s=1},\ldots, D_{t=3,l,s=8}\}$ denote the combined set of the same (mean) densities as calculated in the first simulation experiment and let $D_{l,u}$, $u = 1,\ldots, 36$, denote the uth element. For each length group, the two fish densities at each of the paired stations, $D^1_{l,u}$ and $D^2_{l,u}$, were generated assuming that they both originated from the same lognormal distribution. The new gear was applied to the stations with densities $D^1_{l,u}$, while the old gear was applied to $D^2_{l,u}$.

For the paired hauls design, we do not distinguish between type 2 and type 3 experiments such that CPUEs for the new and old gears, $U^1_{l,u}$ and $V^2_{l,u}$, respectively, were generated based on densities $D^1_{l,u}$ and $D^2_{l,u}$ for $u = 1,\ldots, 36$.

For the new method, we distinguish between type 2 and type 3 experiments. This means that only the stations fished by new gear and the corresponding CPUEs, $U^1_{t,l,s}$, were used in the simulations as the first haul taken in both type 2 and type 3 experiments. The CPUEs from the second haul, $U^2_{t,l,s}$, were generated accounting for the disturbance effect.

The catchabilities and conversion factors used were similar to those in simulation study 1. For a given length group l, the simulation procedure was as follows.

1. Let σ and length group l be specified.
2. $i = 1$.
3. Generate densities $D^1_l(i) = \{D^1_{l,1}(i),\ldots, D^1_{l,36}(i)\}$ and $D^2_l(i) = \{D^2_{l,1}(i),\ldots, D^2_{l,36}(i)\}$ from the lognormal distributions $\{\mathrm{LN}(\ln(D_{l,1}),\sigma),\ldots, \mathrm{LN}(\ln(D_{1,36}),\sigma)\}$.
4. Generate CPUEs

$$U^1_{l,u}(i) \sim \mathrm{NB}(q_{\mathrm{new}} D^1_{l,u}(i)\frac{p}{1-p}, p),\ u = 1,\ldots, 36$$

$$V^2_{l,u}(i) \sim \mathrm{NB}(q_{\mathrm{old}} D^2_{l,u}(i)\frac{p}{1-p}, p),\ u = 1,\ldots, 36$$

For the new method, the CPUEs from the second hauls, $U^2_{t,l,s}$, were generated according to eqs. B2 and B3 in simulation study 1:

$$U^2_{t=2,l,s} \sim \mathrm{NB}(\beta q_{\mathrm{old}} D^1_{l,u}\frac{p}{1-p}, p),\ u = 1,\ldots, 28$$

$$U^2_{t=3,l,s} \sim \mathrm{NB}(\beta q_{\mathrm{new}} D^1_{l,u}\frac{p}{1-p}, p),\ u = 29,\ldots, 26$$

5. Calculate the estimators of the conversion factor $\hat{\gamma}_{1,l}(i)$ for the new method using eq. B4. For the paired hauls design, three estimators were considered:

$$\hat{\gamma}_{3,l}(i) = \frac{\sum_{u=1}^{36} U^1_{l,u}(i)}{\sum_{u=1}^{36} U^2_{l,u}(i)}$$

$$\hat{\gamma}_{4,l}(i) = \left(\frac{U^1_{l,1}(i)}{U^2_{l,1}(i)} \times \ldots \times \frac{U^1_{l,36}(i)}{U^2_{l,36}(i)}\right)^{\frac{1}{36}}$$

$$\hat{\gamma}_{5,l}(i) = \frac{1}{36}\sum_{u=1}^{36}\frac{U^1_{l,u}(i)}{U^2_{l,u}(i)}$$

6. If $i < 10\,000$, $i = i + 1$, go to 3; otherwise stop.

Contrary to simulation study 1, both densities and catches by station were assumed to vary stochastically each time a simulation experiment was performed. This procedure ensured that the effect of density variations by station was included. Relative bias and MSE of the estimators were calculated as in simulation study 1.

The variability of fish density at the paired stations is described by the standard deviation σ, which approximately is the coefficient of variation of the densities. Obviously, the variability increases when σ increases. When $\sigma = 0$ (no variability between nearby stations), all paired hauls provide information useful to estimate γ, whereas in the new design, some effort is "wasted" on estimating the disturbance β. For this case, therefore, the paired hauls design is expected to be superior to the new method. However, the MSE of the paired hauls estimates is expected to increase when σ increases. In contrast, the conversion factor derived from the new method ($\hat{\gamma}_1$) is independent of density. However, as the density by station also varies stochastically for the new design, a slight trend in $\hat{\gamma}_1$ appears in the simulations. In general, it is expected that the two MSE curves intersect for one particular value of standard deviation denoted $\sigma_{0,l}$, as illustrated in Fig. 3. When σ_l is less than $\sigma_{0,l}$, the paired hauls design should be preferred, and when σ_l is larger than $\sigma_{0,l}$, the new method should be preferred. $\sigma_{0,l}$ has been calculated for $\hat{\gamma}_1$ and $\hat{\gamma}_4$ (the two best estimators) for each length group together with the relative bias of the estimators.

Instead of using the log variance, σ, that is input to the simulation study, we have chosen to express the variability by the absolute relative difference between two densities from paired stations, $E_D(\sigma) = E(|D^1_{l,u}/D^2_{l,u} - 1|)$, as this scale is believed to be more interpretable for survey scientists. $E_D(\sigma)$ only depends on σ and it can be shown that

$$E_D(\sigma) = \frac{2}{\sqrt{\pi}}\mathrm{e}^{\sigma^2}\int_0^{\sigma}\mathrm{e}^{-t^2}\mathrm{d}t$$

which is a strictly increasing function of σ.

Appendix C. Determination of the overdispersion parameter in the negative binomial distribution

Consider a given length group and type 2 respective 3 experiments. For convenience, the indices for l and t have been left out. Again let U^i_s be the paired CPUEs for station s and assume that they are distributed as $\mathrm{NB}(v^i_s, p)$, $i = 1, 2$, where

$$v^1_s = q_{\mathrm{new}} D_s \frac{1-p}{p}$$

and

$$v^2_s = \beta q_{\mathrm{new}} D_s \frac{1-p}{p}$$

We know that the conditional distribution $U^1|U$ is beta-binomial (McCullagh and Nelder 1989) with parameters v^1 and v where

$$U^i = \sum_s U_s^i,\ U = U^1 + U^2$$

$$v^i = \sum_s v_s^i,\ i = 1,\ 2$$

and $v = v^1 + v^2$. The distribution is independent of p. The mean and variance of this distribution are

$$E(U^1|U) = U\frac{v^1}{v}$$

and

$$\mathrm{VAR}(U^1|U) = U\frac{v^1}{v}\left(1-\frac{v^1}{v}\right)\left(1+\frac{U-1}{v+1}\right)$$

The variance equation shows that the variance equals the binomial variance multiplied by the overdispersion term od:

$$\text{(C1)}\quad \mathrm{od} = 1+\frac{U-1}{v+1}$$

The present analysis indicates that the distribution of $U^1|U$ is overdispersed. From the estimated value of od, we then want to calculate the parameter p for the original variables U^1 and U^2. In principle, this is impossible because the beta-binomial is independent of p. However, as U is NB(v, p), $E(U) = v\frac{1-p}{p}$ or $\frac{1}{p} = 1+\frac{E(U)}{v}$. Inserting $v = \frac{U-\mathrm{od}}{\mathrm{od}-1}$ obtained from eq. C1, we get

$$\frac{1}{p} = \frac{\mathrm{od}\,\frac{E(U)-1}{U}+1-\frac{E(U)}{U}}{1-\frac{\mathrm{od}}{U}}$$

As U and $E(U)$ are large values compared with 1 and od, we approximately get

$$\text{(C2)}\quad \frac{1}{p} = \frac{E(U)}{U}(\mathrm{od}-1)+1$$

Equation C2 shows that

$$\frac{1}{p}\begin{cases} < \mathrm{od} & \text{for } U > E(U) \\ = \mathrm{od} & \text{for } U = E(U) \\ > \mathrm{od} & \text{for } U < E(U) \end{cases}$$

As $U = E(U)$ is close to the modus of the distribution of U with the largest probability, p was set to $\frac{1}{\mathrm{od}}$. However, the overdispersion in practise can be both smaller and larger than od.

Appendix references

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Landscape controls on boulder-rich, winter habitat availability and their effects on Atlantic salmon (*Salmo salar*) parr abundance in two fifth-order mountain streams

M. Coulombe-Pontbriand and Michel Lapointe

Abstract: We test the effect at river reach and segment scales of landscape controls on the distribution of Atlantic salmon (*Salmo salar*) parr densities, as well as associated variations in boulder (diameter ≥ 256 mm) abundance and potential overwintering habitat. This study encompasses data from 45 km of fifth-order mainstem channels along two neighbouring river catchments in the Gaspé region, Québec. At both scales, winter habitat availability was correlated with boulder availability. At the river segment scale (1–5 km), parr densities significantly correlated ($P < 0.05$) with boulder availability along the Bonaventure River, which presented significant intersegment variations in boulder abundances. In contrast, segment-scale boulder and parr abundances were uniformly low along the Petite Cascapédia River. At the reach scale (600 m), positive but less strongly significant boulder – parr abundance correlations were observed in both the Bonaventure and Petite Cascapédia rivers. Spatial variations in boulder abundances in these systems reflected variations in the degree of channel to valley walls coupling and imposed channel formative shear stresses. In similarly boulder-poor segments with comparable fry abundances, parr abundances were significantly greater along the Bonaventure than the Petite Cascapédia River, possibly because of the presence in the former system of nearby boulder-rich refugia segments.

Résumé : Nous avons vérifié à l'échelle de la section et du segment de rivière les effets des facteurs de contrôle du paysage sur la répartition des densités de tacons du saumon de l'Atlantique (*Salmo salar*), de même que les variations associées dans l'abondance des blocs rocheux (diamètre ≥ 256 mm) et dans l'habitat potentiel d'hiver du saumon. Notre étude rassemble des données provenant de 45 km de chenaux principaux de cinquième ordre dans deux bassins versants voisins de Gaspésie, Québec. Aux deux échelles, la disponibilité de l'habitat d'hiver est en corrélation avec la disponibilité des blocs rocheux. À l'échelle du segment de rivière (1–5 km), la densité des tacons est en corrélation significative ($P < 0,05$) avec la disponibilité des blocs rocheux le long de la Bonaventure qui présente des variations significatives d'abondance des blocs d'un segment à un autre. En revanche, le long de la Petite Cascapédia, les abondances de blocs et de tacons sont uniformément faibles à l'échelle du segment. À l'échelle de la section (600 m), il existe des corrélations positives, mais moins fortement significatives, entre les abondances des blocs rocheux et de tacons, tant dans la Bonaventure que dans la Petite Cascapédia. Les variations spatiales de l'abondance de blocs rocheux dans ces systèmes sont le reflet des variations du degré de connexion entre le chenal et les murs de la vallée et des contraintes de cisaillement imposées qui sont impliquées dans la formation du chenal. Dans des segments similaires pauvres en blocs rocheux qui possèdent des abondances comparables d'alevins, l'abondance de tacons est significativement supérieure le long de la Bonaventure que le long de la Petite Cascapédia, probablement à cause de la proximité dans la première de segments riches en blocs rocheux qui servent de refuges.

[Traduit par la Rédaction]

Introduction

Because large cobble and boulder substrate is an essential component of rearing, sheltering, and overwintering habitat of Atlantic salmon (*Salmo salar*) parr (ages 1^+ and above, here designated 1^{++}), it has been speculated that in some river systems, long reaches with little substrate coarser than smaller cobbles could be limiting to parr production (Chapman 1966; Cunjak 1996). If this hypothesis is correct, subtle differences in valley and river geomorphology that affect substrate coarseness could account for occasionally striking contrasts in parr abundance between rivers located in the

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same region that otherwise have similar spawning habitat, primary productivity, and overall watershed characteristics (size, hydrology, and geological history). This paper investigates associations at two spatial scales among patterns in 1^{++} parr densities, boulder (particles ≥256 mm) abundances, and availability of winter habitat (defined through a combination of hydraulic and sedimentological criteria) over 45 km of two adjacent fifth-order rivers with similar salmon 0^{+} fry but very contrasted 1^{++} parr abundances. The paper also analyses the nature and spatial scales of the landscape factors that control coarse substrate and par abundance patterns.

The importance of coarse substrate for parr habitat has been confirmed in a number of studies. While feeding during the rearing season, 1^{++} parr generally maintain position behind protruding, larger cobble and boulder size "home stones" at the proximity of fast currents where drifting prey are more abundant (Wankowski and Thorpe 1979; Rimmer et al. 1984; Morantz et al. 1987). Cobbles and boulders also provide cover for sheltering from predation (Valdimarsson and Metcalfe 1998). As water temperature cools down below about 8 °C in autumn and winter, juvenile salmonids switch to a winter, nocturnal behaviour, seeking shelter in large interstices under large substrate during daytime and emerging during nighttime (Cunjak 1988; Heggenes et al. 1993; Contor and Griffith 1995). The effect of a scarcity of coarse substrate on limiting winter habitat and carrying capacity of streams may be particularly strong in regions where winter conditions are severe, such as eastern Canada (Cunjak 1996; Cunjak and Therrien 1998; Cunjak et al. 1998).

Benda et al. (1992) demonstrated the importance of large-scale landscape processes (and their control on the location of boulder-rich and boulder-poor reaches) in structuring salmonid habitat in a river basin. Rice et al. (2001) argued that in mountain valleys, major mesoscale units of stream habitat variation are sedimentary links, defined as lengths of river valley over which substrate size, substrate mobility, and channel geometry change in a predictable way between node points where coarser sediments are reinjected into the river by tributaries or valley side landslides. However, relationships between coarse substrate availability and parr abundance, although well documented at the microscale level (through habitat preferences within a given reach), have not been statistically tested at larger scales across river reaches and river segments. In the study systems, a reach is defined as a river section measuring 600 m, i.e., measuring approximately 10 times the bankfull channel width and including approximately two pool–riffle units (Frissel et al. 1986). Channel segments are defined as the longest river sections (1–5 km or 10–60 times the bankfull channel width) with relatively homogeneous geomorphic character in terms of discharge, substrate calibre, channel slope, and degree of lateral valley confinement.

Two major hypotheses are tested in this paper. The first hypothesis is that along a total length of 45 km of two fifth-order mainstem salmon rivers with similar 0^{+} fry abundances, 1^{++} parr density patterns correlate at the river reach and segment scales with the availability of coarse (boulder) substrate and winter habitat. The second hypothesis is that in these two mountain valley systems, spatial variations in boulder abundance and winter habitat can be predicted from geomorphic variables reflecting the degree of lateral confinement by valley walls (valley to channel width ratio, bankfull shear stresses, and channel shifting rates) and associated lateral supply of coarse substrate.

Materials and methods

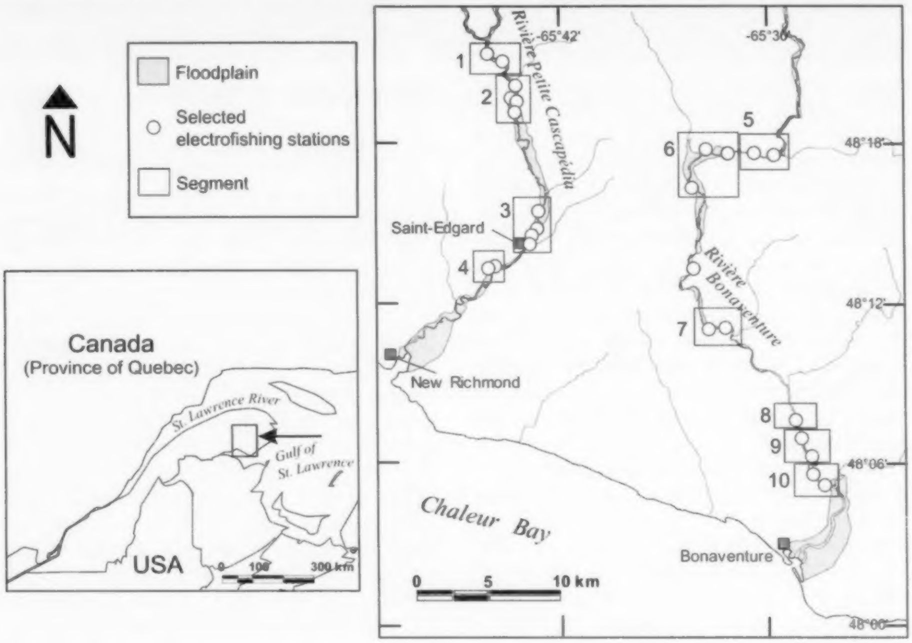
Study sites

The study was performed in the main stems (Fig. 1) of two neighbouring cobble-bed rivers of the Gaspé Peninsula, Province of Quebec, with contrasting abundances of parr and adult Atlantic salmon. The Petite Cascapédia River produces, on average, about three times fewer adult salmon per square kilometre of accessible potential rearing habitat than does the Bonaventure River (Perron 1992*a*, 1992*b*; Dorais 2000), despite intensive stocking of salmon fry and smolts over the 1980–1997 period. Electrofishing inventories performed by the Quebec Wildlife Agency (Société de la faune et des parcs du Québec, FAPAQ) over 10 years show that average observed 1^{++} parr abundance is seven times lower in the Petite Cascapédia than in the Bonaventure River main stem, although, overall, both river main stems have comparable quality of spawning habitat (based on spawning zone grain size analysis and redd surveys; Coulombe-Pontbriand and Lapointe 2004) and similar 0^{+} fry abundance (FAPAQ, 308 chemin Saint-Edgar, New Richmond, QC G0C 2B0, Canada). In particular, redd counts (done over 2 years) were uncorrelated with either fry or parr counts for the following years at either spatial scale. Differences in food production have been discounted as the probable cause of these contrasts in parr abundances; juveniles found in the Petite Cascapédia River are larger at a given age than those found in the Bonaventure River (Dorais 1997). The dominant fish populations in the study rivers are Atlantic salmon, anadromous and resident brook trout (*Salvelinus fontinalis*), and slimy sculpin (*Cottus cognatus*).

The discrepancy in parr (and adult) abundances is puzzling considering the proximity and general similarity of the adjacent watersheds. Both rivers flow in a general north to south direction from the Shickshock Highlands of the Appalachian Mountains into Chaleur Bay, although fault lines locally disrupt their southerly course. The watersheds cross similar bedrock lithological units, which trend east to west. Folded Ordovician to Devonian mudstone, conglomerate, and limestone underlie the rivers' main stems, whereas granitic and ophiolitic rocks dominate in the headwaters near the Shickshocks Highlands. South of the Shickshocks Highlands, the mainstem valleys of these two rivers are incised 200–300 m into the forested plateaus of an old Appalachian erosion surface. Interfluves are relatively flat and the valleys are either flat bottomed with steep walls or V-shaped. Although interfluves are actively being logged in their upper reaches, lower watersheds are predominantly forested. Discontinuous sandy to gravel–boulder terraces of fluvio-glacial origin locally border the floodplains.

The two fifth-order systems are of comparable scale and both show relatively high hydraulic energy levels (70–150 $W \cdot m^{-2}$ of unit flow power at bankfull stage) and high gravel–cobble transport rates. Bankfull channel widths and mean channel gradients in the two study systems are similar: 60 m and 0.20% for the Petite Cascapédia River and 73 m and 0.23% for the Bonaventure River. Although flow-

Fig. 1. Map of study sites along the Petite Cascapédia River and Bonaventure River, Gaspé Peninsula, Province of Quebec, Canada.



ing in a generally wider valley, discharge in the Petite Cascapédia River is about two thirds of that in the Bonaventure River. At the town of St-Edgard, located 9.5 km from its mouth, the Petite Cascapédia River drains a watershed of 1340 km^2 and has a mean flow of 33 m^3·s^{-1} (1961–1981). By comparison, the Bonaventure River drains a 1910-km^2 watershed and has a mean flow of 52 m^3·s^{-1} at the gauging station 17 km from its mouth (1965–1983). Both rivers have unregulated snowmelt-dominated hydraulic regimes.

Glacial history

The strongest impacts of the Pleistocene continental glaciations affecting the study rivers until 10 000 years BP were some valley avulsions rerouting river sections and the deposition of a fluvio-glacial esker along the lower Bonaventure River (in segment 8, Fig. 1). In its lower part, the Bonaventure River is incising into bedrock, apparently shaping a young valley after being rerouted from its ancient path at an unknown point in time, possibly during early Pleistocene glaciations.

Filling of the lower valleys with fluvial and deltaic deposits induced by a marine transgression (high relative sea levels) during a period of isostatic land depression shortly after deglaciation is attested by deltas perched 47 m above sea level 7 km upstream of both rivers' estuaries. The clearly visible braided character of fluvio-glacial terraces found locally along valley segments suggests larger sediment supply and greater peak discharges during the paraglacial period (immediately after the glacial period, Church and Ryder 1972) than during present-day conditions. After isostatic uplift and a decline in relative sea level, the study rivers gradually incised into their paraglacial valley fills, mobilizing large quantities of material that was evacuated to the sea or stored in terraces and floodplains or are still being reworked by rivers. In the current conditions, boulder alluvium does not get transported far from local sources and a significant quantity of large boulders found along the bed of the contemporary river systems is inherited from a more energetic paraglacial environment. Contemporaneous sources of coarse material input in the river systems are the local erosion of fluvio-glacial terraces, bedrock valley walls, and the supply from some tributaries

Channel morphology

There is a typical trend, from low-order to high-order streams in mountainous drainage basins, both in the transition of channel morphologies (Montgomery and Buffington 1997) and in the corresponding salmonid habitat structure (Benda et al. 1992). Typically, streams gradually change from alluvium supply-limited, high-energy headwater channels with boulder bed materials (cascade and step–pool types) to transport-limited, low-energy channels with sandy beds (displaying dune–ripple bedforms). In supply-limited channels, the high transport capacity (shear stresses) of typical high flows rapidly evacuates the limited supply of relatively fine alluvium, exposing a lag of boulder-size material that is mobilized only during rare high flow events. On the contrary, plentiful fine alluvium supply exceeds the lower transport capacity (shear stresses) of transport-limited channels, mantling the bed with fine bed material that is mobilized well below bankfull discharge. In between these states, mountain streams transition through moderate-energy cobble–gravel systems with near-bankfull mobilization threshold (plane bed and pool–riffle types).

The nature of sediment supply likewise exhibits a typical downstream transition. Headwater channels are strongly "coupled" with their narrow, confining valleys in a sediment budget sense and directly receive irregular inputs of sediment materials of all sizes from flanking hillsides through landslides, valley wall erosion, and debris flows. In contrast, downstream channels are relatively uncoupled from their valley sides as they migrate relatively freely on extensive floodplains and mainly and steadily receive large amounts of finer, better-sorted material from fluvial transport processes (Montgomery and Buffington 1997). In many fluvial systems such as the fifth-order main stems of the Petite Cascapédia and Bonaventure rivers, this idealized pattern of downstream change in the geomorphic characteristics of the channels (with theoretically monotonic trends in sediment supply, channel shear stress levels, and degree of confinement) is locally perturbed by a variety of mechanisms. Major basin-scale geomorphic events (valley rerouting, glaciation, and stream pirating) and geological controls (faulting and differential bedrock resistance) can impose valley confinement and lateral sediment coupling in channels of any stream order (Montgomery and Buffington 1997). Rice et al. (2001) described frequent, predictable disturbances in the regular downstream fining of bed material and a decrease in channel energy along mountain river systems associated with point-source recruitment zones of coarse sediment at some tributaries and landslide sites.

Although the two study rivers generally flow over their own gravel–cobble alluvium, in a number of places, they are also confined by valley walls or controlled vertically by outcrops of resistant bedrock (Fig. 1). Most of the Petite Cascapédia River valley is relatively wide (typically 0.5–1 km wide), with channel uncoupled from valley walls, whereas much of the Bonaventure River valley is substantially narrower (under 0.5 km) and displays a higher degree of valley side coupling (Table 1). The Petite Cascapédia River exhibits segments with pool–riffle and forced pool–riffle morphologies (Table 1). In addition to these two channel morphologies, the Bonaventure River also displays higher-energy, more supply-limited plane-bed and bedrock-dominated segments (Montgomery and Buffington 1997). Thus, the main stems of the study rivers overall display contrasting valley and channel morphologies. The possible associations between these geomorphic contrasts and the puzzling discrepancy in 1^{++} parr densities between the study rivers despite similar 0^{+} fry abundances are the main focus of this investigation.

Parr abundance record and selection of study sites

Data on relative 0^{+} fry and 1^{++} parr abundance at a network of fixed sites were available from a database collected from 1986 to 1999 by the Quebec Wildlife Agency, FAPAQ, Direction Régionale de la Gaspésie. This database is intended to monitor relative differences in densities rather than absolute abundances and includes the results of electrofishing counts performed in July or August at the same location over 9 years along the Bonaventure River (1986–1993 and 1999) and over 8 years along the Petite Cascapédia River (1986–1988 and 1990–1994). Sampling was performed in 100-m^2 open stations (5 m wide by 20 m long), typically spaced 1 km apart, well away from zones where fry or smolts were stocked. No stocking of 1^{++} parr occurred in the study rivers during electrofishing years. One electrofishing pass of 15-min duration was performed each year at every station. The same operator accomplished electrofishing in any given year. Hand-held fishnets were used. Stations were located on riffles and bar margins or in runs where flow depth was under 50 cm and velocities under 1 $m \cdot s^{-1}$. These conditions where selected to ensure representation of optimal parr habitat in each stream site (M. Dorais, FAPAQ, 124 1^{re} Avenue Ouest, Sainte-Anne-des-Monts, QC G4V 1C5, Canada, personal communication). Despite the interannual variability observed in electrofishing data at any site, such long-term data sets can yield useful insights into systematic spatial patterns in parr abundance by allowing us to compare multiyear average parr density scores within and between study systems. Because parr strongly compete for prime summer feeding habitat, consistently low parr abundances within such habitats indicate that factors other than feeding habitat availability within the adjoining channel stretch restrict parr survival or site fidelity.

In total, 25 of these electrofishing stations were selected for physical habitat analyses, 12 on the Petite Cascapédia River and 13 on the Bonaventure River, as index sites of the 45 km where detailed river surveys were conducted (Fig. 1). Stations were selected to satisfy two criteria: to be representative of the range of observed parr abundances and to allow grouping of a few consecutive stations into river segments that are homogeneous in terms of channel and valley morphology (relative valley width, slope, boulder density, and channel migration rates). At each of these 25 FAPAQ stations, mean annual parr densities were calculated over the years of record. Although the average interannual coefficient of variation in parr densities sampled at any given station was high (84%), across sites, this interannual variation had a common temporal component so that some stations had generally higher parr abundance, whereas others had consistently lower parr abundance. For example, five out of 12 stations on the Petite Cascapédia River and four out of 13 stations on the Bonaventure River consistently displayed lower parr abundances when compared with all electrofishing counts on the same river (two-tailed t tests, $P < 0.05$). In addition, one station displayed significantly higher than average parr counts in the Petite Cascapédia River, whereas two did in the Bonaventure River ($P < 0.10$).

Measurement of boulder density and overwintering habitat

Boulder density and potential overwintering habitat were measured along 600-m-long reaches centred on the FAPAQ stations, usually extending to adjacent riffle zones both downstream and upstream. This chosen reach length was consistent with other studies (Quinn and Peterson 1996; Piegay et al. 2000). Reaches were then grouped into 1- to 5-km-long river segments with relatively uniform morphology, each containing one to five reaches. Since segments were defined to be relatively uniform, the average of within-segment reach-scale values for parr abundance, winter habitat, and boulder density scores was assigned to each river segment. Such detailed surveys cover 45 km of channel length along the two rivers. Although comparably detailed habitat surveys could not be conducted along the whole main stems of both rivers, cursory habitat observations (and FAPAQ juvenile abundance data) were also available for

Table 1. River segment morphology in the Petite-Cascapédia River (P.C.) and Bonaventure River (Bon.).

River	Segment	Channel type	Valley width (km)	Relative valley width	Channel to valley side coupling
P.C.	1	Forced pool–riffle	0.14	4.1	Moderate to high
P.C.	2	Pool–riffle	0.55	15	Low
P.C.	3	Pool–riffle	0.53	12	Low
P.C.	4	Forced pool–riffle	0.29	6.1	Moderate
Bon.	5	Bedrock	0.08	1.5	High
Bon.	6	Pool–riffle	0.58	9.6	Moderate to low
Bon.	7	Forced pool–riffle	0.23	4.1	Moderate to high
Bon.	8	Plane bed–bedrock	0.09	1.6	High
Bon.	9	Plane bed	0.17	2.3	High
Bon.	10	Forced pool–riffle	0.23	3.6	Moderate to high

Note: Channel type is assessed according to the classification scheme proposed by Montgomery and Buffington (1997). Relative valley widths are given as the ratio between valley width and river width.

river sections adjacent to detailed study segments depicted (Fig. 1).

As a quantitative measure of substrate coarseness, the average number of boulders per square metre of channel bed was preferred over the standard bulk-sampling protocol, as described in Church et al. (1987). The method representatively measured reach-scale boulder availability at the bed surface and therefore relates directly to coarse parr habitat quantity. The number of boulders was counted within the boundaries of 1-m^2 bed surface quadrats evenly distributed at 5-m intervals along transects. A particle touching the borders of the quadrat was counted only when more than 50% of its area was included inside the quadrat. The same operator consistently performed boulder counts, thus avoiding differences resulting from operator bias. Within reaches, transects extending between the limits of channel banks were taken every 50 m for a total of 13 transects per 600-m reach. Within the channel parts located between reaches of a given segment, transect spacing was 200 m. Inaccessible deep pools and unusually high-velocity thalweg zones were avoided (5–10% of the cases). The number of boulders counted in each 1-m^2 parcel was first averaged by transect and then by reach and by segment over 45 km of channel length.

Potential overwintering habitat was assumed to depend on the proximity of both suitable nighttime and daytime winter habitats. As water temperature cools down in autumn and winter, juvenile salmonids switch to a winter behaviour, seeking shelter under large-cobble and boulder clusters typically located near the thalweg, the deepest zone of the channel during daytime (Heggenes 1988; Heggenes et al. 1993; Contor and Griffith 1995). Bed surface areas dominated by large cobbles (>128 mm) or boulders with clean (nonembedded) interstices were considered suitable daytime winter habitat. During winter nights, about two thirds of juvenile salmonids migrate to shallow, low-velocity areas along stream banks to feed (Heggenes et al. 1993; Whalen and Parrish 1999). Nighttime winter habitat was defined as channel bed areas with velocities under 20 $cm \cdot s^{-1}$ and depths under 60 cm based on published overwintering microhabitat preferences (Table 2). Potential overwintering habitat was defined as an area of the channel bed meeting nighttime habitat criteria in proximity to cobble–boulder clusters, either within the area itself or within a 5-m-wide zone surrounding that area. Mean water column velocities were estimated at 60% of the depth with a Pygmy current meter. Substrate was visually classified as suitable or unsuitable for winter habitat. Finally, the total estimated area of potential overwintering habitat in a study reach was divided by the total wetted channel area of that reach, measured during low summer flows, to obtain the percent area of available overwintering habitat.

The presence of a thick ice cover made the assessment of potential winter habitat during the winter season impracticable at the scale of the present study. The total channel area meeting the hydraulic criteria for overwintering habitat was surveyed in the field during summer 2000 low flows assuming, as a first approximation, that the patterns of hydraulic conditions observed in a reach during summer low flows were broadly similar to those occurring during low winter flows. Summer 2000 low discharges were only slightly higher than mean winter discharges: respectively 45% and 30% of mean annual discharge or 9.7% and 6.5% of mean peak discharge (Fig. 2). Ice cover, however, could modify the hydraulic conditions. Low-velocity zones situated at the channel margins are prone to freezing into black or frazil ice (Cunjak et al. 1998). Ice cover presents an increased resistance to water flow and a subsequent reduction in velocities. An overall increase in water elevation can therefore be expected. Thus, in winter, low-velocity nighttime habitat under ice cover may in some reaches be more extensive than indicated by our summer surveys, especially in sectors far from frazil sources.

Measurement of bankfull shear stress and channel migration rates

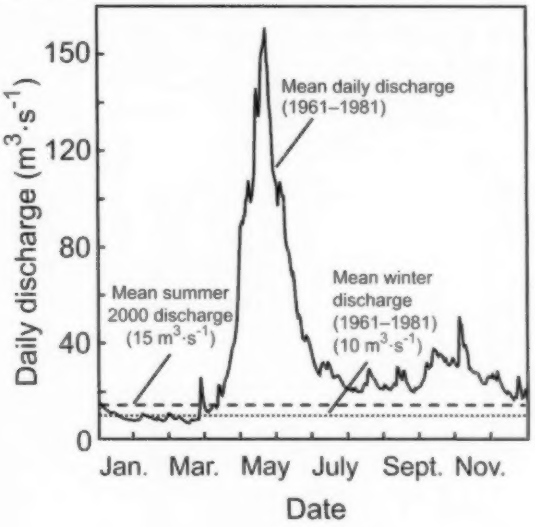
Shear stress represents the average force per unit area applied to bed material and channel forms within a reach by running water and is a common index of energy available for transporting coarse sediment. Shear stresses at or near bankfull discharge are typically associated with a threshold of significant mobilization of bed material and the moulding of the channel perimeter in higher-order plane-bed and pool–riffle channels with cobble–gravel beds (Carling 1988).

Table 2. Review of the microhabitat preferences of Atlantic salmon juveniles during winter.

		Nighttime	
Reference	Daytime, substrate (cm)	Depth (cm)	Velocity ($cm·s^{-1}$)
Rimmer et al. 1984	20–40	24–36	<10
Cunjak 1988	16.8–23	40.9–48.9	38.7–45.7
Griffith and Smith 1993	>20	<50	—
Whalen and Parrish 1999	—	15–60	~20

Note: Daytime wintering habitat is defined as cobble–boulder substrate and nighttime habitat as areas of low velocities (<20 $cm·s^{-1}$) and low depths (<60 cm).

Fig. 2. Hydrograph of the Petite Cascapédia River (1961–1981). Mean daily discharges are displayed as a solid line, mean winter discharge (1961–1981) as a dotted line, and mean summer discharge of 2000 (the survey year) as a broken line.



Segment-integrated bankfull shear stress (τ_o) was estimated using Du Boys equation (Lapointe et al. 2000):

$$\tau_o = \rho g D S$$

where ρ is the water density, g is gravitational acceleration, D is channel depth, and S is the channel energy slope. Channel and floodplain topography was surveyed in detailed using a total station and a laser level tied to high-precision control points georeferenced with a differential ground positioning system. Assuming uniform flow conditions, the energy slope of the channel at bankfull stage (S) was approximated by the floodplain slope along the channel margins (Lapointe et al. 2000). Measuring the channel depth (D) for bankfull conditions at riffle crests and averaging by river segment eliminated biases related to difficulties in surveying the deepest pools. This increase in between-segment comparison reliability was, however, gained at the price of a slight but systematic underestimation of the true segment-integrated bankfull shear stresses.

Channel migration was measured at 3-m precision by comparison of multiyear aerial photographs using a Zoom-Transferscope. Bank erosion rates were calculated at the apex of individual channel bends using two series of aerial photographs with a 29-year time span (1963–1992). Photograph scale varied between 1 : 10 000 and 1 : 15 000. Valley bottom width (or floodplain width where high terraces are present) was measured on cross sections from 1 : 20 000 topographical maps aided with stereo-airphoto interpretation.

Results

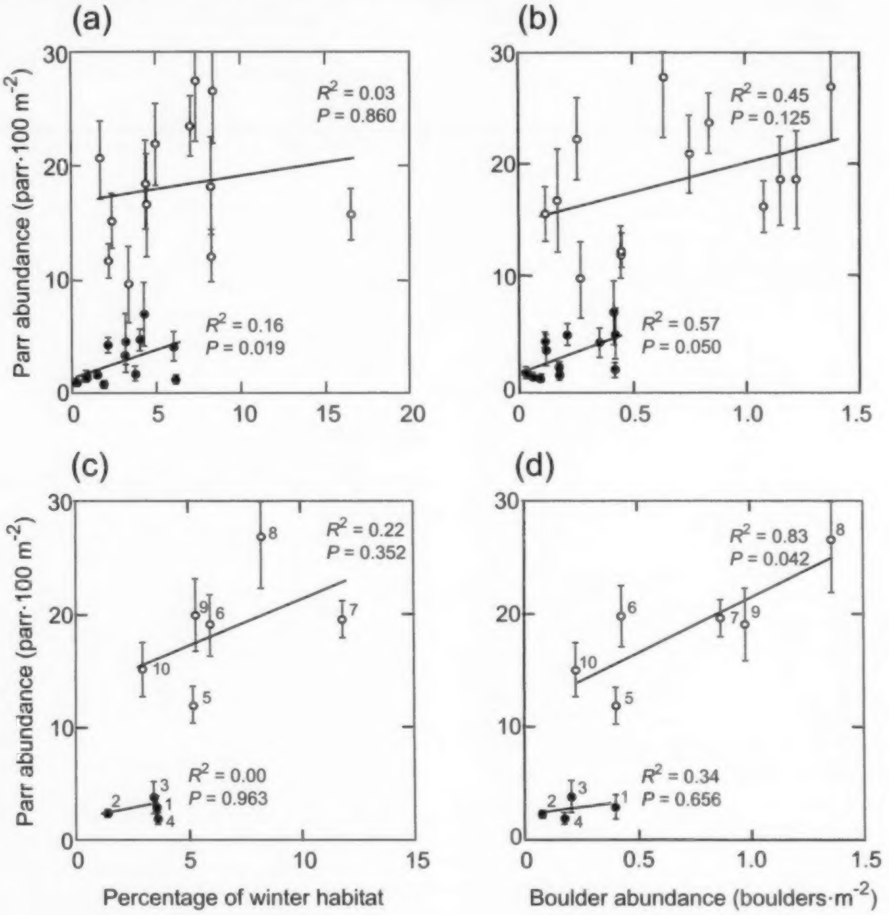
Parr abundance, boulder density, and overwintering habitat

The study reaches of the Petite Cascapédia and Bonaventure rivers present contrasted parr abundance, as well as boulder density and winter habitat scores (Fig. 3). Relative parr abundance scores, in the range 0.6–4.6 parr·100 m^{-2} in the Petite Cascapédia River, are significantly less than the 6.6–26 parr·100 m^{-2} observed in the Bonaventure River (t test, $P < 0.01$). Overall, there are significantly fewer boulders and less winter habitat in the Petite Cascapédia River (0.2 boulder·m^{-2} and 2.8% winter habitat) than in the Bonaventure River (0.7 boulder·m^{-2} and 6.2% winter habitat) (t test, $P < 0.01$).

Pooled data from both rivers (Fig. 3) suggest significant relationships between parr abundance, abundance of boulders, and availability of winter habitat at both scales ($P < 0.01$). However, these pooled associations are mainly driven by the large differences in parr and boulder abundance between the two rivers (Fig. 3, open and solid circles). Relationships between parr abundances and availability of habitat within one population (i.e., inhabiting the main stem of one river) can more easily be interpreted. At the segment scale, parr abundance correlates significantly with boulder abundance in the Bonaventure River ($R^2 = 0.83$, $P = 0.042$) (Fig. 3*d*). Within the Petite Cascapédia River, this relationship is not significant at the segment scale (note that the number of segments in this river, four, is very low). Data trends (Fig. 3) (confirmed by covariance analysis, $P < 0.01$) also reveal a river-specific effect exerted by availability of coarse habitat: even reaches (or segments) with similar low boulder abundances, parr densities are significantly higher on the Bonaventure than on the Petite Cascapédia River (Figs. 3*b* and 3*d*).

Parr abundance does not correlate significantly at the 5% level with the availability of overwintering habitat in either river at either scale, although empirical trends in the relationship are positive (Figs. 3*a* and 3*c*). Winter habitat, as quantified in this study, does not provide a gain in explana-

Fig. 3. Atlantic salmon parr abundance against percentage of wetted channel usable as winter habitat and against boulder abundance at (*a* and *b*) reach and (*c* and *d*) segment scales. Solid circles are data from the Petite Cascapédia River (1986–1993, 1999); open circles are data from the Bonaventure River (1986–1988, 1990–1994). Parr abundances are multiannual averages from electrofishing counts. Vertical bars represent ±1 SE of the mean of multiannual parr abundance.



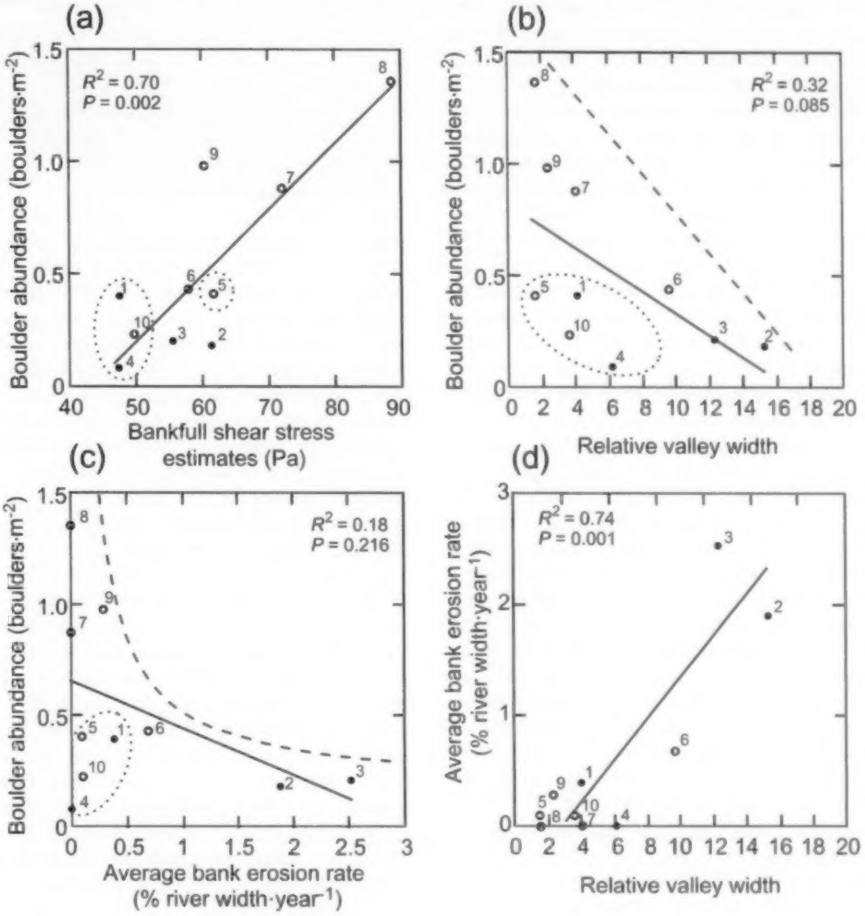
tion of summertime parr abundance patterns over that provided by boulder density. Note that in the study systems, the two predictors are not independent. The availability of overwintering habitat is significantly correlated with boulder abundance at either scale (R^2 = 0.64 at the reach scale, R^2 = 0.78 at the segment scale). Marginal low-velocity zones are equally widespread along both study systems (*t* test, P = 0.909) and the winter habitat scores mostly reflect variations in the availability of coarse substrate for daytime hiding.

Geomorphic controls on the spatial distribution of boulders

The observed patterns of boulder abundances reflect complex, segment-scale differences in the degree of lateral valley confinement and associated variations in coarse substrate supply, channel energy level, and degree of lateral channel instability (Fig. 4). Overall, the Petite Cascapédia River segments display greater valley to river width ratio (i.e., are less laterally confined (Fig. 4*b*)) (P = 0.058) and have lower boulder abundance (P = 0.018), lower formative shear stress levels (P = 0.051), and greater channel shifting rates (P = 0.096) than the Bonaventure River segments. Low significance values are due to small number of river segments (10). At the segment scale, boulder abundance is strongly related to channel bankfull shear stress levels across the two systems (R^2 = 0.70, P < 0.01) (Fig. 4*a*).

Although the relative valley width (Fig. 4*b*) and rates of bank erosion (Fig. 4*c*) do not correlate significantly with boulder abundance at the 5% level, these variables appear to affect the maximum measured boulder abundance, as seen by the shape of the data cluster envelope of the scatterplots (dashed lines in Figs. 4*b* and 4*c*). In effect, in segments within narrow valleys (relative valley width less than 4 (Fig. 4*b*)), a large range of boulder densities are measured depending on the local context, but all laterally unconfined (uncoupled) segments display low boulder density (Fig. 4*b*). Similarly, although the variations in boulder density are considerable in narrow valley segments were the river is laterally confined and thus has low bank erosion rates (Fig. 4*c*), boulder density is very low in segments that are free to me-

Fig. 4. Segment-scale relationships between geomorphic variables of the channel and the valley in the Petite Cascapédia River (solid circles) and Bonaventure River (open circles). Relative valley width is the ratio between the floodplain width and the bankfull channel width. Broken lines represent the trend in maximum boulder abundance (envelope) as a function of (*b*) relative valley width and (*c*) bank erosion rates. River segments confined by valley walls and presenting low boulder abundance are circled with dotted lines in Figs. 1*a*, 1*b*, and 1*c*).



ander in wide valleys and consequently display high lateral instability. When only the segments with the largest boulder densities are considered (segments 2, 3, 6, 9, 7, and 6), maximum boulder abundance decreases strongly with increasing valley bottom width and bank erosion rates ($P < 0.05$). These relationships appear linear for valley bottom width (Fig. 4*b*) and nonlinear for bank erosion rates (Fig. 4*c*).

The majority of coarse sediment sources identified in the Petite Cascapédia and Bonaventure rivers correspond to zones of lateral erosion of the bedrock valley walls and boulder-rich glacio-fluvial terraces emplaced under paraglacial conditions. Overall, such sources of coarse sediment are more frequent along the Bonaventure River than along the Petite Cascapédia River. The most striking source of coarse substrate is observed in segment 8 (with mean boulder density of 1.3 boulders·m^{-2}) where the Bonaventure River is completely hemmed in in a very narrow valley section (valley to channel width ratio of 1.6) while eroding a boulder-rich terrace of subglacial (esker-type) fluvio-glacial origin (Fig. 1). Segment 8 has the highest mean interannual parr density (27 parr·m^{-2}) of either system.

Discussion

Associations between boulder and parr abundance

Along the Bonaventure River, where boulder densities vary greatly between segments, parr abundance values are significantly higher in boulder-rich segments compared with segments with fewer boulders. The same association is not detectable across the four segments of the Petite Cascapédia River, were boulder and parr are quite low everywhere. However, at the reach scale, where variations are stronger and data points more abundant, these relationships also show positive but weakly significant trends in both systems.

The data point to a strong "river-specific effect": i.e., reaches having similar low boulder and winter abundance contained remarkably lower parr abundance in the Petite Cascapédia than in the Bonaventure River. FAPAQ electrofishing data confirm that parr abundances are very low everywhere along the Petite Cascapédia main stem, including the sections between our study segments. This discrepancy suggests that large-scale factors other than boulder or winter habitat abundance at a site might be responsible for the lower parr abundances observed in the main stem of the Petite Cascapédia River (when compared with similarly boulder-poor sections of the Bonaventure River).

The notable dearth of older juveniles in the Petite Cascapédia River cannot be explained by poorer fry production or fry survival conditions. Although midsummer water temperatures are 1–2 °C cooler in the Petite Cascapédia than in the Bonaventure River main stem, the two systems overall have comparable quality of spawning habitat and trophic conditions (Coulombe-Pontbriand and Lapointe 2004). The FAPAQ electrofishing data reveal that mean fry abundances are not statistically different between segments of the two rivers (based on 10 years of surveys at 12 and 13 stations for the respective rivers, $P < 0.05$).

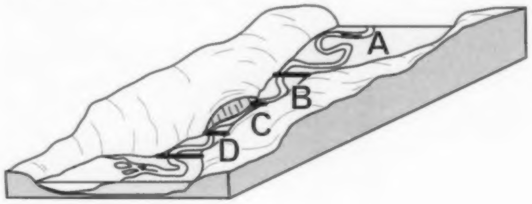
Although we have no direct migration observations to conclusively test this hypothesis, the migration of parr from boulder-poor into available boulder-rich refugia with greater overwintering survival in the Bonaventure River appears to be a likely explanation for the river-specific effect. Cunjak and Randall (1993) reported substantial parr migration during winter and greater site fidelity in stream sections more adequate for overwintering habitat in three streams of eastern Canada. Field surveys along the Bonaventure River show that boulder-poor but parr-rich segments 5, 6, and 10 all lie within a few kilometres downstream of boulder-rich segments or tributaries. In contrast, because of the extreme dearth of coarse substrate along the Petite Cascapédia River, even parr migrating long distances along the main stem of the Petite Cascapédia River would unlikely find substantial quality coarse winter habitat. This appears to impose severe limits on survival of larger parr in this system.

If this interpretation is correct, in rivers with severe winter stress to juveniles, occasional boulder-rich channel units may be necessary to support high parr abundances in boulder-poor valley segments, even when situated kilometres away. This supports the view that the relative arrangement and interconnections, at the valley segment scale, between river main stem and tributary habitats with different physical characteristics need to be considered in future juvenile salmon production models.

Geomorphic controls on the spatial distribution of boulders

Although boulder abundance and segment-integrated shear stresses were strongly correlated, it would be erroneous to conclude that segment-average shear stresses causally explain the distribution of boulder abundance. These variables are mutually interdependent in alluvial rivers: the input in significant quantity of coarse substrates into a reach generally results in steeper slopes and high shear stresses. A reduction in the degree of lateral confinement tends to operate on both variables in the same direction: it diminishes coarse sediment supply from valley sides and allows for lower overall channel energy. We propose a simple landscape model for the main controls on the distribution of boulder-rich parr habitat in confined mountain valley systems with only minor tributary streams, comparable with the study systems (Fig. 5).

Fig. 5. Schematic diagram of the river segment types observed in the Petite Cascapédia and Bonaventure rivers: site A, large semialluvial; site B, narrow semialluvial; site C, bedrock dominated; site D, narrow semialluvial with downstream control.



In narrow coupled valley sections (site B, Fig. 5), widespread erosion of valley walls and marginal terraces produces numerous contemporary sources of coarse sediment and generally results in coarser bed material. Whether coarse stones observed in the study rivers are derived from contemporary valley-side point sources or are inherited from reworking of paraglacial deposits is difficult to ascertain. According to field observation and theory, boulders originating from limited point sources, such as landslide sites along valley walls and terraces, usually have limited downstream dispersal distances. Through selective transport and abrasion, bed material typically exhibits size fining downstream of its source, if there is no other significant lateral sediment sources (Rice 1999). Moreover, such downstream fining is more pronounced for coarser particles than for finer particles (Seal and Paola 1995) and should be most rapid for boulder-size particles. It is reasonable to assume that mobilization of boulders inherited from high-energy paraglacial ancestral rivers is relatively infrequent under the lower energy conditions of present-day rivers. Boulders present in the channel since paraglacial times presumably rest as lag or static armour deposits that contribute to overall channel coarseness where formative shear stresses are strong enough to prevent burying by finer bed material (i.e., Montgomery and Buffington 1997).

Segments affected by downstream hydraulic controls (such as sea level) that impose low slopes (site D, Fig. 5) tend to maintain lower shear stresses and remain boulder poor, even where laterally confined. Such downstream controls explain the large variations in boulder abundances observed in confined segments. For example, segments 1, 4, 5, and 10 in this study occur in relatively narrow sections of valleys but plot well below the maximum boulder abundance lines (Fig. 4*b*). Segments 1, 4, and 10 are also the three segments with the lowest shear stress conditions (Fig. 4*a*). It is noteworthy that formative shear stresses in these segments are constrained by lower slopes reflecting the downstream control imposed by sea level (segments 4 and 10) and of an outcrop of resistant bedrock across the channel bed (at the end of segment 1). Such transport-limited segments accumulate fine cobble and gravel transported through the upstream reaches. We suspect that in such lower slope segments, fine gravel can rapidly bury any large alluvium produced by the erosion of the valley walls and terraces or inherited from paraglacial material.

Wider, uncoupled valley segments (site A, Fig. 5) present few contemporary sources of coarse substrate. Channels meandering within these wider valleys are less frequently in contact with the valley walls, resulting in fewer lateral sediment sources. In addition, these segments have the freedom to meander, resulting in greater sinuosity, gentler channel gradient, and lower formative shear stresses. Channel conditions tend to rapidly become transport limited, resulting in finer bed material. Finally, in these wide valleys where channel migration rates are high and shear stresses are lower, the probability is high that occasional boulders in transport through these reaches become trapped in pools over time (and eventually buried as a consequence of channel migration).

Bedrock-dominated channels (site C, Fig. 5) seem to obey a different set of rules than do alluvial channels (Thinkler and Wohl 1998). In segment 5, with moderate shear stresses (Figs. 4*a*, 4*b*), the bedrock surface was essentially boulder free. Apparently, any exposed coarse alluvium would rapidly be transported on this smooth bedrock surface and become trapped in deep bedrock pools or deposited in downstream reaches.

Field observations suggest that boulder clusters recently recruited to the channel bed from localized contemporary sources generally display more large interstices than lag boulders inherited from paraglacial material and imbedded in armoured riverbeds. This suggests that the most favourable context ensuring regular distribution of adequate boulder-rich habitat along a stream involves frequent coarse sediment sources along channels with adequate shear stresses to rework these inputs (supply-limited conditions). In the study sites, these conditions were best met in narrow, coupled valleys bordered by coarse terraces of fluvio-glacial origin and in confined valleys where the active erosion of valley walls provides regular sources of interstice-rich boulder clusters.

Management implications

Possible habitat management implications of these findings need to be further tested. Given the preference of 1^{++} parr for habitat containing abundant coarse sediment, it has long been suspected that inserting boulder clusters in boulder-poor reaches may represent a simple and effective solution for increasing salmonid production in rivers (Meyer and Griffith 1997; Cunjak et al. 1998; Valdimarsson and Metcalfe 1998). Although our interpretation of the Bonaventure River data in the Discussion suggests that the separation between boulder-rich sections can be of the order of kilometres in rivers of this size, the optimal spacing of such boulder emplacement needs to be investigated as a function of the physical characteristics of the river.

Furthermore, there is a need to test the long-term cost effectiveness of artificial boulder emplacements as a means of enhancing salmon runs (rather than temporarily redistributing parr among reaches). In mountain valleys, segments that lack coarse particles typically correspond to wide, uncoupled valley segments presenting low lateral constriction and relatively high migration rates. Given the overall high mobility of underlying cobble–gravel substrate, occasional boulder clusters introduced in such an environment may, within a few years, migrate to pools or become buried by point bar accretion as the river shifts, thus becoming inaccessible to parr. To enhance long-term stock levels, the time scale during which artificial boulder emplacement provides useful habitat needs to significantly exceed the generation time scale of the salmon population, unless frequent boulder replacement can be contemplated.

In summary, the present study supports the idea that a lack of boulder-rich habitat may limit Atlantic salmon 1^{++} parr survival in a stream affected by severe winter conditions and highlights the importance of the occasional occurrence of boulder-rich channel units. In the fifth-order study streams, hydraulic and sedimentary conditions ensuring coarse habitat are best met in narrow, coupled valleys segments. These findings also highlight the significance of the link discontinuity concept discussed by Rice et al. (2001): geomorphological discontinuities created by points of coarse material recruitment along the river continuum can create variations in boulder abundance over short distances within channel units that are comparable with variations observable between mountain streams of different orders and can enhance system-level production. Future fish production studies need to account for coupling between fish habitat requirements, channel processes, and valley geomorphology at a relevant scale for watershed management.

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PERSPECTIVE / PERSPECTIVE

Early life history studies of prey–predator interactions: quantifying the stochastic individual responses to environmental variability[1]

Pierre Pepin

Abstract: Laboratory evidence shows that growth and survival of larval fish are strongly affected by variations in prey and predators; field evidence, in general, does not. This discrepancy may be partly due to the mismatch of scales at which manipulative and observational studies are conducted, or perhaps field studies are somehow not detecting the variable component of the larvae or their environment. I discuss potentially important variable features of fish larvae and their environment and show how mean values can be misleading. Using data from several field studies dealing with the growth and mortality of radiated shanny (*Ulvaria subbifurcata*) larvae, I illustrate how observational programs can miss important variation. I show evidence of how differences among individuals may lead to varying responses to fluctuations in prey availability. I also discuss issues concerning the level of variability in environmental conditions that may be described by standard survey methods used in the study of larval fish. The examples are intended to serve as illustration of the need to better describe the underlying stochastic structure of environmental conditions to understand early life dynamics.

Résumé : Bien que les données de laboratoire démontrent que les variations des proies et des prédateurs affectent fortement la croissance et la survie des larves de poissons, les données de terrain ne le font généralement pas. Cette contradiction peut en partie être due à un désaccord d'échelle entre les observations de terrain et les expériences de laboratoire; ou alors, les études de terrain n'arrivent pas pour une raison ou une autre à détecter la composante de la variabilité chez les larves de poissons et dans leur environnement. Certaines des caractéristiques variables d'importance potentielle chez les larves de poissons font l'objet d'une discussion; il est aussi démontré que l'utilisation des valeurs moyennes peut induire en erreur. Des données provenant de plusieurs études de terrain de la croissance et de la mortalité des larves de l'ulvaire deux-lignes (*Ulvaria subbifurcata*) servent à illustrer comment des plans d'observation peuvent ne pas déceler de la variation importante. Il y a, de plus, des données qui montrent comment les différences entre les individus peuvent entraîner des réactions différentes aux fluctuations de la disponibilité des proies. Il est aussi question des problèmes concernant le niveau de variabilité des conditions de l'environnement qui peut être décrit à l'aide des méthodes courantes d'inventaire utilisées dans l'étude des larves de poissons. Ces exemples se veulent des illustrations de la nécessité de mieux décrire la structure stochastique sous-jacente des conditions de l'environnement afin de comprendre la dynamique de la vie des jeunes poissons.

[Traduit par la Rédaction]

Introduction

The dynamics of early life stages of fish are the result of prey–larva–predator interactions. Predicting variations in development or mortality requires an understanding of the probabilities of such interactions, how they are affected by the physical environment or by the biological factors that affect the aggregation and dispersal of animals, and consequently the overlap among trophic levels. From egg to metamorphosis, larvae undergo significant changes in body size and form and consequently interact with a changing suite of prey and predator species. Laboratory studies clearly demonstrate that larvae grow faster when food is more abundant (Leggett and Deblois 1994) and that a wide variety of organisms prey on fish eggs and larvae (Bailey and Houde 1989). However, field studies have generally failed to show clear

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evidence that prey–predator interactions play a significant role on development and survival of fish eggs and larvae. Some researchers have argued that general patterns in development and size-dependent mortality rates among species and ecosystems show patterns consistent with expectations based on trophic relationships (Peterson and Wroblewski 1984; Houde 1989; Pepin 1991). Others have suggested that estimates of mortality have not taken into account the potential contribution of losses due to transport and that the apparent size-dependency simply reflects a bias caused by the duration of the study and the physical dimensions of the region of interest rather than prey–predator interactions (Taggart and Frank 1990; Helbig and Pepin 1998). The emergent question is whether our approaches provide an adequate description of the environmental conditions faced by early life stages.

Any sequence of observations of a cohort of larval fish represents samples from a probability distribution that is the result of a series of stochastic processes. First, a sample of n larvae represents a draw from a probability distribution of possible densities (e.g., negative binomial, Power and Moser 1999). Second, eddies affect the dispersal and aggregation of plankton and thus affect the probability that we are observing a similar parcel of water (the same population) from one period to the next. Finally, the number of larvae surviving within this parcel of water depends on the density of predators encountered, which will vary from parcel to parcel according to the local distribution of predator densities over the drift path. If we were to consider growth rather than survival, then the distribution of prey densities becomes key to determining prey–predator interactions. The ambit of both the larva and its predator(s) determines the scale over which we need to determine the probability distributions of encounters. The product of these probability distributions represents the error structure from which we draw our observations and against which we should contrast them if we are to develop an understanding of the factors that affect larval dynamics. Thus, the observation of different cohorts or patches of larvae that are subject to different environmental conditions in which dispersal or the distribution or aggregation of predators (or prey) are likely to be different may not be drawn from identical probability distributions of possible outcomes. As a result, contrasting survival (or growth) with the average density of predators (or prey) during each observation period will not be the appropriate basis for comparison. Instead, it is the likelihood of observing an outcome relative to our expectation of the distribution of probable outcomes that should be the basis for assessing the possible link between cause and effect. To gain this understanding requires that we be able to predict or quantify changes in the underlying form of the probability distributions of prey–predator encounters.

It may appear that the problem of studying the dynamics of larval fish is an intractable one because of all the underlying sources of variability. The fact that the problem appears to be intractable relates more to how we have chosen to represent the environment of larval fish instead of asking ourselves what is needed to understand prey–predator interactions. The same could be stated for any other element of the pelagic food web, because the same factors that affect prey–predator interactions for larval fish will come into play for other organisms. Larval fish may in fact provide a unique tool for studying the operation of marine ecosystems: (*i*) their production is often restricted in time and space, which can allow more effective tracking of cohorts and their environment given the right tools; (*ii*) otolith microstructure can provide a history of individuals and can allow us to determine which fraction of the population is more likely to survive; (*iii*) their rapid and significant development from hatch of metamorphosis provides a perspective on prey–predator interactions over a wide portion of the pelagic food web. Some challenges of studying fish larvae include the following: (*i*) they are relatively rare, in contrast to other zooplankton, and thus estimates of abundance can be less precise than for more abundant organisms; (*ii*) unbiased samples of the population become more difficult to obtain as larvae develop because of avoidance of nets; (*iii*) developing growth histories from otolith microstructure is both time consuming and costly. However, the knowledge of pelagic marine ecosystems gained from research into early life history dynamics can provide new insights of how changes in the distribution and movement of different taxa or trophic levels can impact on population dynamics and productivity.

The discrepancy in the scale of observations between laboratory and field studies (Fig. 1) may be an important factor in the difficulty to link variations in growth and survival with fluctuations in ecosystem structure. Experimental manipulations of trophic interactions can only be carried out in small static enclosures, with their duration limited by the loss of individuals. In contrast, field surveys of population dynamics are based on coarse resolution of the spatial structure of the environment and limited by a vessel's ability to cover the geographic region of interest. Furthermore, field studies are often carried out over relatively short time intervals, thereby limiting the range of environmental variability encountered. If we consider how these differences affect our description of the environment of a larva, the limitations of our approaches may become more apparent. For example, a larva that grows from 3 to 20 mm in length will search approximately 0.01–1 $m^3 \cdot day^{-1}$ (Fig. 2). Ultimately, this represents the grain size (sample unit) that we would hope to describe the level of variation in the prey environment among individuals. However, our typical representation of the feeding environment is based on standard plankton nets that sample prey by integrating samples over ~5–100 m^3, which provides us with an average prey concentration over a broad scale relative to the larva's feeding ambit. More importantly, these sample units are then used to represent environmental conditions over ~10^6–10^{10} m^3 (Fig. 2), based on the typical resolution (station spacing) used in broadscale surveys. Even if we were to consider depth-stratified sampling and interpolation of observations between stations, typical field studies involve smoothing environmental conditions over scales that are 7–10 orders of magnitude greater than that perceived by a larva. Given such differences in the scale of observations and interactions, can researchers provide an adequate representation of the probability of encounters between prey and predator (whether the larva is the predator or prey)?

While studying the dynamics of larval fish, we cannot know or represent all of the factors that govern prey–predator

Fig. 1. Area and duration of field and laboratory studies of the dynamics of larval fish. The open circles at the right of the plot show the overall duration of field studies in relation to the area surveyed. The error bars along the abscissa represent the area represented by each station of the survey, and the bars along the ordinate represent the time interval between surveys. The data are from the studies summarized by Helbig and Pepin (1998). The grey lines and symbols at the left of the plot summarize the scales of laboratory studies dealing with predation (horizontal solid line) and feeding and development (vertical solid and broken lines) based on the data by Paradis et al. (1996) and Benoît et al. (2000). The area of laboratory studies is based on the assumption that the experimental tanks had a depth of 1 m. The dotted lines at the top of the plot represent the range of life span from hatch to metamorphosis for most species (Benoît et al. 2000).

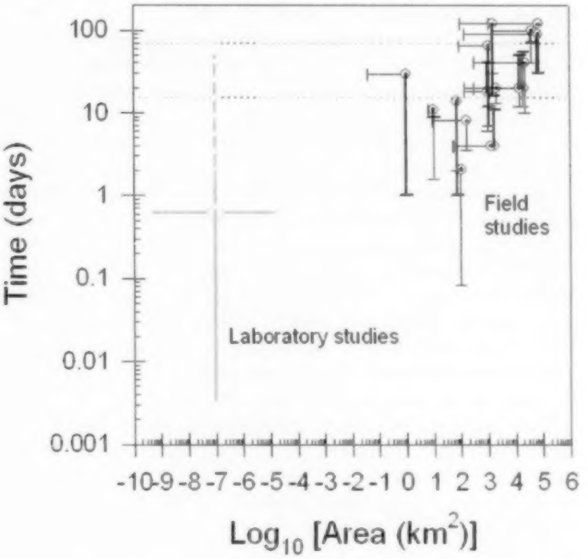
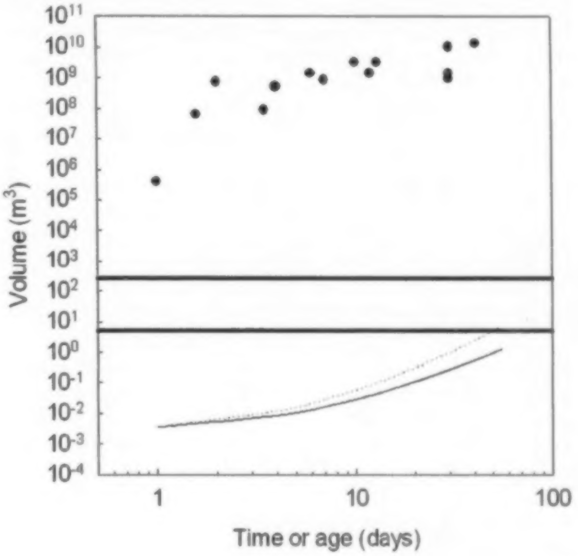


Fig. 2. Schematic diagram showing the age-dependent daily (thin solid line) and cumulative (dotted line) volume searched by a larval fish in relation to age based on a swimming speed of one body length (BL) per second and an encounter radius of one BL for a hypothetical larval growing from 2 to 21 mm in length at a growth rate of 0.33 mm·day^{-1}. The thick solid lines represent a typical range of volumes filtered by plankton nets while estimating microzooplankton densities during field studies (J. Dower, School of Earth and Ocean Sciences, University of Victoria, Victoria, BC V8W 2Y2, personal communication). Solid circles show the area represented by each station during the field surveys summarized by Helbig and Pepin (1998) in relation to the interval between surveys.



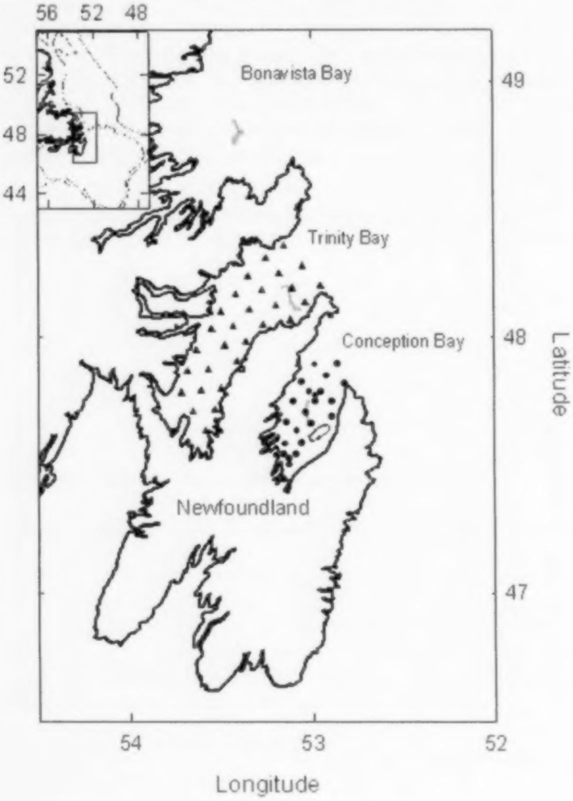
interactions, but we can attempt to identify some of the key elements that will affect our ability to predict the outcome of those interactions. As a starting point, consider whether the state of individuals of a given age or size influences their potential variations in growth. If differences exist, then the response to changes in the environment will be affected by the distribution of individual states within the population. Beyond the individual, variations in the structure of the environment faced by the larvae represents the next issue to consider.

In this perspective, I argue that the study of prey–predator interactions that affect larval fish requires a significant change in our approach to sampling the marine environment in the methods by which we describe the distribution of organism density (abundance) and in the assumptions we make about our knowledge of the early life stages of fish. I first show how differences among individuals may lead to varying responses to fluctuations in the environment. I present arguments that differences in growth rates among cohorts may emerge because underlying structure or patchiness of the larva's prey field changes. Finally, I argue that standard surveys may not be adequate to describe the overall variability in environmental conditions. To illustrate these points, I use data from several field studies dealing with the growth and mortality of larval radiated shanny (*Ulvaria subbifurcata*). The examples are intended to show the types of questions that should be asked of our approaches and studies rather than as a thorough review of the underlying questions that remain unanswered.

Data

The data used in this perspective were collected as part of three studies of radiated shanny larvae carried out in coastal Newfoundland waters (Fig. 3). One study consisted of following three patches of larvae, using daily sampling in distinct environments (Dower et al. 2002; Pepin et al. 2002) in July 1997, whereas the other two consisted of repeated surveys whereby the local population was sampled at 5- to 7-day intervals over a 2-week period in Conception (Pepin et al. 2003) and Trinity (Baumann et al. 2003) bays during July and August of 1998 and 2000, respectively. In all surveys, station spacing was set at ~5–8 km, which is approximately the Rossby radius (a measure of the size of the dominant eddy in the region). This resulted in 9, 23, and 29 stations in

Fig. 3. Map of eastern Newfoundland, Canada (inset), showing study sites for which data are presented. The drift track during patch studies (solid grey lines) were carried in 1997, whereas population studies of Conception (solid circles) and Trinity (solid triangles) bays were carried out in 1998 and 2000.



the patch, Conception Bay, and Trinity Bay studies, respectively. At each station, larvae were sampled using a single oblique tow (0–40 m) with a Tucker trawl and microzooplankton were sampled with a single vertical tow of a fine mesh net. Abundance of juvenile and adult capelin (*Mallotus villosus*), the dominant planktivorous fish in the region, was estimated using a calibrated, hull-mounted, split-beam 38 kHz transducer and an EK500 echosounder system. Larval growth patterns were determined from sagittal otoliths.

Drift histories were simulated using a three-dimensional, eddy-resolving model (Davidson et al. 2001), with a realistic coastline geometry and bottom topography and forced with spatially homogenous winds (Baumann et al. 2003; Pepin et al. 2003). To assess the origin of larvae associated with a given station at the time of sampling, we used backward projections of particle tracks. Particles were uniformly seeded 500 m apart and assigned to sampling stations after a 6-day simulation only if they were located within 3 km of the station location within the model domain. The projected drift of larvae sampled from a particular station was assessed by seeding 1000 particles within a radius of 1 km around every sampling station occupied on a given sampling day and projecting their position after a 6-day simulation period.

Individual growth patterns

An individual's growth is influenced by genetics, environment, and luck. When dealing with early life stages of fish, some important questions concerning growth rates are (*i*) to what degree does an individual maintain its rank with regard to the scope for growth throughout development, and (*ii*) is the potential variation in growth rates the same for all individuals? These questions deal with probabilities of events. In the first case, the issue is where does an individual lie within a cohort's distribution of growth rates, and what is the probability that this may change with time. Whether the latter varies depending on the state of the individual is the point of the second question.

Variations in growth among cohorts or individuals is one of the most widely studied aspects of early life history. Otolith microstructure provides an effective tool by which the growth history of an individual fish can be described (Campana and Nielson 1985). Most analytical approaches of growth have focused on a parametric (deterministic) characterization in patterns among cohorts (e.g., Campana 1996) or individuals (e.g., Maillet and Checkley 1991). The functional relationships that emerge from such studies effectively describe the average growth rates and not the differences among individuals. Furthermore, it is clear that an individual's growth rate shows autocorrelation (Mosegaard et al. 1988; Pepin et al. 2001), which places constraints on the degree of variation one is likely to observe within an individual in response to changes in the environment.

Consider the distribution of individual growth histories of larval radiated shanny (Fig. 4). Nonparametric local density estimators (Davison and Hinckley 1997) were used to describe the probability distribution of increment width in relation to age and to determine whether fast- or slow-growing individuals responded to changes in food availability in a similar manner. In this species, increment width is a good reflection of the instantaneous growth in weight (Pepin et al. 2001). A cross section of the probability distribution of observations at any age reveals that growth rates follow a skewed distribution in which most of the individuals have growth rates that are below the population mean. At this point, it is important to note that selective loss from the population, because of the strong interaction between growth and mortality (predator risk or starvation), will affect the perceived distribution of growth rates. The surviving larvae are the uncommon ones, which may preclude reporting some of the most common histories. By contrasting the fate of individuals that are at the upper (85th–95th percentiles of the population) and lower (5th–15th percentiles of the population) ends of the distribution of daily increment widths, it becomes evident that the magnitude and rate of variation in growth rate is dependent on the state of the individual and on the age at which we observe it. For example, the growth rate of larvae at 5 days old appears to have relatively little impact on the probability distribution of growth rates at older ages (Fig. 5). For both fast- and slow-growing portions of the population, the scatter (distance between the 10th and 90th percentiles) in growth rates beyond age 5 days quickly

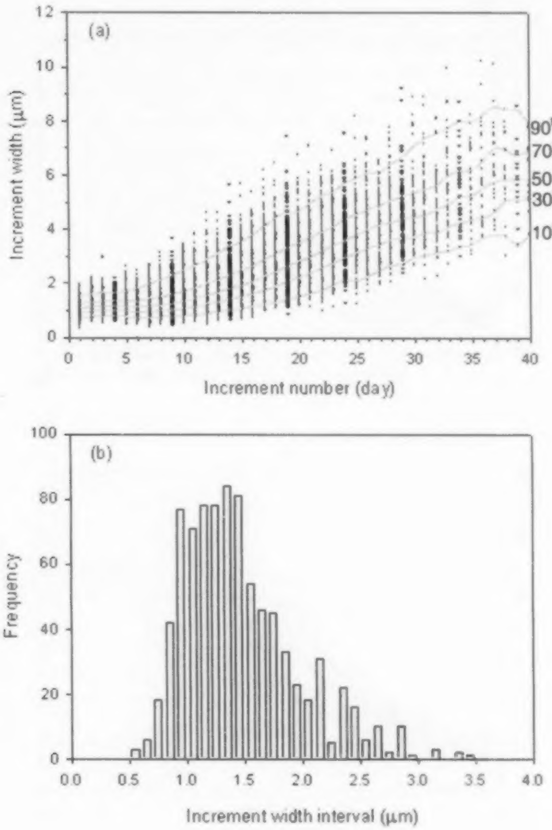


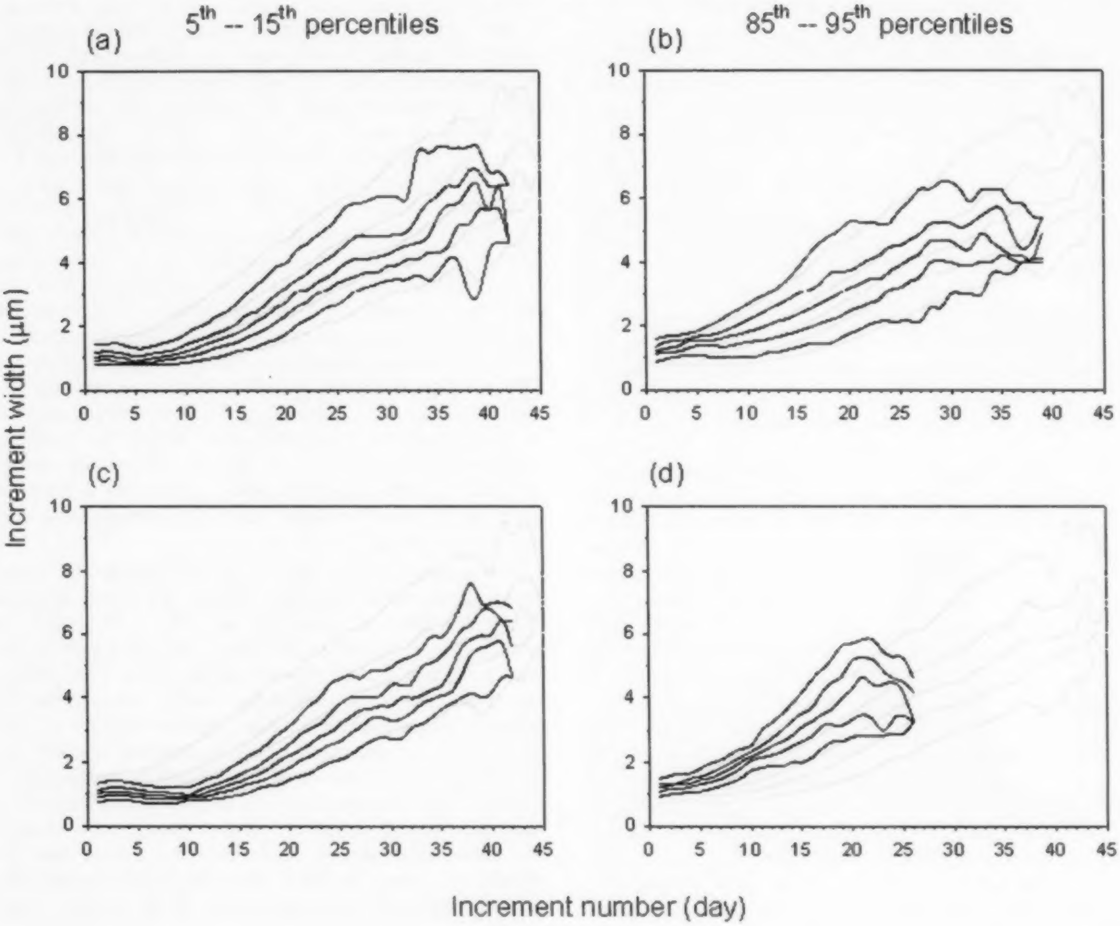
Fig. 4. (*a*) Distribution of individual daily growth increment widths in relation to age for 987 larval radiated shanny. The solid grey lines show the 10th, 30th, 50th, 70th, and 90th percentiles of the distribution estimated using local nonparametric density estimators with a bandwidth of 2 days. (*b*) Frequency distribution in individual increment widths for 10-day-old larvae.

reaches that of the population (Fig. 5*a*, 5*b*). At this point in life, growth rates are still somewhat of a lottery. However, there is a marked contrast in the distribution of future growth rates when we consider fast- and slow-growing portions of the population at 10 days old (Fig. 5*c*, 5*d*). Slow-growing individuals at 10 days are unlikely to achieve high growth rates, whereas the opposite is true for fast-growing individuals. Furthermore, the variance in individual growth rates is greater and increases more rapidly for fast-growing individuals than for slow-growing ones. As larvae become older, the increasing scope for growth of fast-growing individuals may reflect greater responsiveness to variations in food availability, whereas this may be less apparent in slower growing animals because the probability for changes is substantially reduced.

The consequence of variations in the responsiveness of individual larvae becomes important when we attempt to reconstruct the drift and environmental history for a sample of the population (for example, see Baumann et al. 2003). In individuals that were aged 10 days or less 1 week before capture, there is very little apparent effect of the median food concentration encountered at that time on the change in the distribution of growth histories (Fig. 6*a*). Whether individuals had high or low growth rates, the subsequent distribution of growth rates is similar and not significantly affected by particle concentration. Furthermore, there is little evidence that prior growth rates had a substantial impact on growth at capture 1 week later (i.e., there appears to be limited autocorrelation). If it had, then the distribution would be tightly constrained along the one-to-one line. In individuals older than 10 days 1 week prior to capture, food concentration has a marked effect on individuals that had high growth rates, with significantly greater variation appearing in individuals that encountered low versus high prey densities, whereas individuals with low growth rates are unaffected (Fig. 6*b*) (significance was evaluated using a randomization test based on 500 iterations). However, individuals with high growth rates make up a small proportion of the population, given the skewed distribution of individual growth rates. The patterns described above indicate that we cannot identify any significant predictability in growth rates during the early life and that the later state of an individual depends to a large degree on random events during the early larval phase. When we contrast cohorts, the question then becomes whether cohorts with high growth rates have a greater proportion of individuals with good luck versus those cohorts with low average growth rates.

Foraging by larval fish is generally modeled as a random process whereby encounters follow a Poisson distribution where the average encounter rate can be affected by prey abundance and factors that affect the movement of water past a larva (e.g., turbulence) (Werner et al. 1996; MacKenzie et al. 1994). The underlying form of the probability distribution of encounters remains constant and the variance is always equal to the mean, a key assumption in many studies of prey–predator interactions. (It should be noted that many studies of the statistical aspects of animal distributions show that measures of variability tend to change with changes in the mean (Gaston and McArdle 1994; Power and Moser 1999), in contrast to the Poisson distribution often used to simulate prey–predator encounters). If we consider that the distribution of daily growth increments is a reflection of the distribution of encounters, albeit somewhat smoothed because of the lag in the physiological response to variations in foraging success, then we would expect all individuals to fall along a single, general, variance-to-mean relationship for individual growth rates. Whether average prey concentration or other factors increase encounters, the mean and variance should follow the form of the underlying encounter process, assuming that the probabilities of attack and capture are unaffected by those factors. Differences among cohorts would be in the distribution of individuals along this general relationship: a fast-growing cohort would be characterized by a greater proportion of individuals with high and variable growth rates, and vice versa. To investigate this hypothesis, I estimated the mean and variance in individual growth rates during the first 15 days of life for all larvae from the three studies (five cohorts) that serve as the basis for this perspective. Increment widths were standardized across all cohorts, as in Pepin et al. (2001). Fifteen days was selected because by this age, individual growth characteristics are highly cor-

Fig. 5. Cumulative probability distribution of individual otolith increment widths in relation to age (*a*) for larvae in the lower 5th–15th percentiles (black lines) of the population (grey lines) at 5 days old; (*b*) for larvae in the upper 85th–95th percentiles (black lines) of the population (grey lines) at 5 days old; (*c*) for larvae in the lower 5th–15th percentiles (black lines) of the population (grey lines) at 10 days old; (*d*) for larvae in the upper 85th–95th percentiles (black lines) of the population (grey lines) at 10 days old. In each panel, the lines for the subset of larvae and the population show the 10th, 30th, 50th, 70th, and 90th percentiles of the distribution.

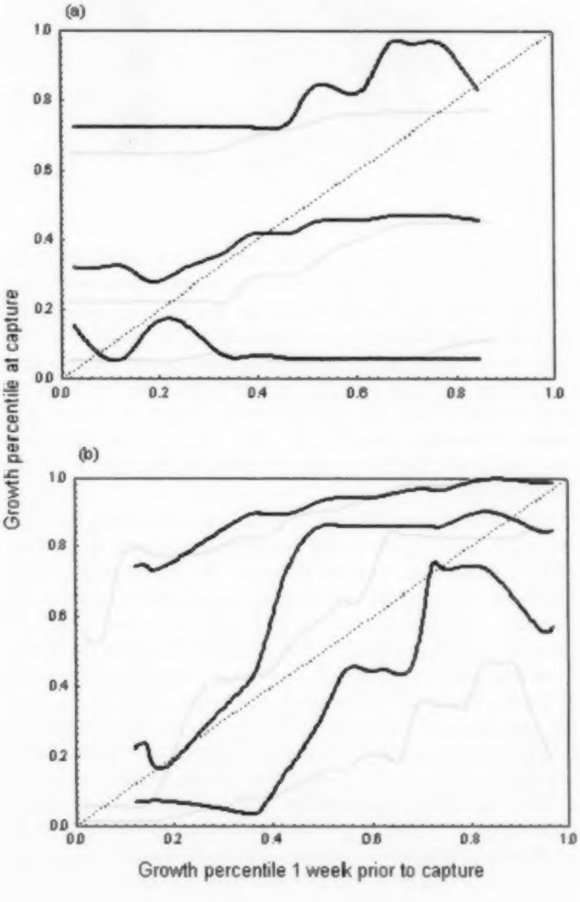


related with subsequent growth, as well as to provide a balance in the number of specimens available for analysis. An analysis of covariance of the variance-to-mean relationship of individual growth rates among cohorts shows that cohorts with different average growth rates follow parallel relationships where the elevation was significantly different ($F_{[4,557]} = 6.83$, $P < 0.001$) but where the slope of the relationships was not significantly different among cohorts ($F_{[4,557]} = 0.11$, $P > 0.5$). The slope is also significantly less than 1:1 ($t = 10.8$, $P < 0.001$). Cohorts with slower average growth rates exhibited a higher variance in individual growth rates than in faster growing cohorts (Fig. 7). The contrast in the intercepts among cohorts with different average population growth rates suggests that the environment is perceived differently by the individual larvae, so that to achieve equal growth rates, individuals in poor feeding environments are likely to have more highly variable daily growth rates than if feeding in a good feeding environment. An individual's growth rate integrates its functional feeding response and the distribution of prey encounters over time. If we believe that the functional response is determined by age or size, the implication is that to understand variations in growth rates among cohorts, our sampling of the feeding environment must be able to characterize the structure and patchiness of encounters at a scale relevant to larval fish. Without such information, contrasting growth rates across cohorts is unlikely to detect important differences in the effect of prey availability on larval growth rates (Dower et al. 2002).

Environmental conditions

We face two fundamental issues in trying to represent the environmental conditions encountered by larval fish. The first is associated with how we sample the ecosystem to represent prey–predator conditions over space and time. The second is associated with the time required for the larva or the population to respond to changes in environmental conditions and

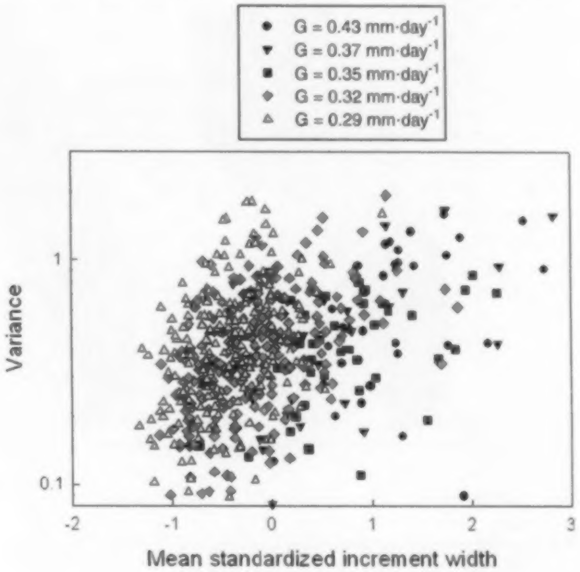
Fig. 6. Cumulative probability distribution showing the 10th, 50th, and 90th percentiles of the distribution of growth rates at capture in relation to growth rates 1 week prior to capture for larvae that encountered median microzooplankton concentration <30 prey·L^{-1} (grey lines) and those that encountered >30 prey·L^{-1} (black lines) for individuals that were (*a*) <11 days old 1 week prior to capture and (*b*) >11 days old 1 week prior to capture. The dotted line shows the one-to-one relationship. To determine if the change in the scatter in relation to growth rate was significantly different, a randomization test based on 500 iterations was applied. The median prey concentration for each individual was estimated from cumulative probability distribution of microzooplankton density (counts·m^{-3}) based on the observations from the nearest station sampled 1 week prior to capture using a backward reconstruction of drift histories.



the certainty with which we can reconstruct or predict the probable environmental conditions.

The way the biological environment in which larval fish live is sampled places limitations on our effectiveness to describe the prey–predator interactions that determine vital rates. As indicated in the Introduction, our sampling averages much of the variability that a larva perceives. However, a more fundamental question is how to determine what proportion of the underlying variability in biological conditions is being represented by our sampling strategies. Put another way, how much of the variability in prey abundance occurs at spatial scales smaller than the nearest sampling sites in a survey?

Fig. 7. Variance-to-mean relationship of standardized individual growth increments during the first 15 days of life for larvae from cohorts with different average growth rates. Each cohort represents larvae from one of the five sites included in this analysis.



Studies of the relationship between larval fish and their environment are typically conducted at regional population scales. Although some studies are conducted at small local scales (e.g., Taggart and Leggett 1987) or on distinct patches (Davis et al. 1991; Murdoch and Quigley 1994; Pepin et al. 2002), extrapolating the results of those observations to the population level has seldom been carried out. At the regional scale, the distance between stations is typically 5–20 km. From such collections, it is possible to generate regional representations of the distribution of zooplankton prey on which the larvae feed. For example, the sampling conducted in Trinity Bay in 2000 yielded three views of the distribution of microzooplankton (Fig. 8), which changes in relation to changes in hydrographic conditions and local production (Baumann et al. 2003). However, the estimation of the standardized variogram, which provides a measure of the similarity among sites as a function of the distance separating them, shows that the variability among nearest sampling sites (stations) is approximately 50%–60% of that observed among the most distant sites (Fig. 9). Although one could fit a functional form (model) to the estimated variogram, the key implication is that there is considerable variability in plankton abundance at scales smaller than the nearest sites, which we use to represent the prey field available to larvae (Fig. 9). If our surveys do not provide an effective characterization of the patchiness of prey at the regional scale, how can we expect to understand how variations in prey availability affect early life dynamics? One might be tempted to argue that these results are simply specific to the region (or because of the study design). However, the more important point is that similar analyses have not been performed as

Fig. 8. Spatial distribution of microzooplankton density (counts·m^{-3}) during three consecutive surveys of Trinity Bay on days (*a*) 200, (*b*) 206, and (*c*) 211.

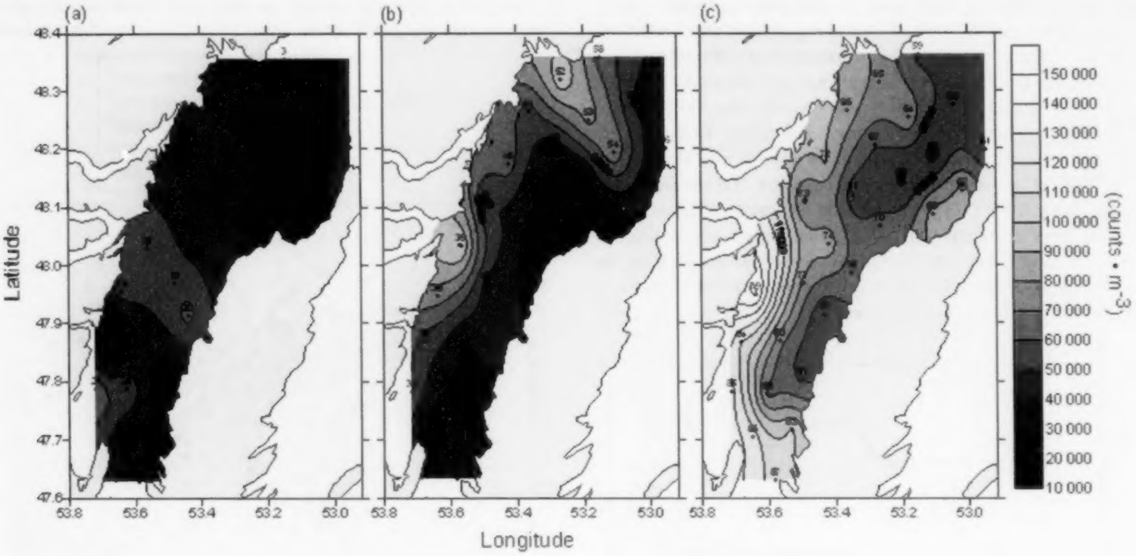
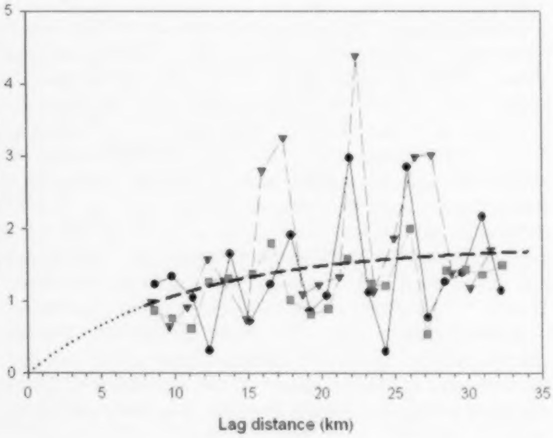


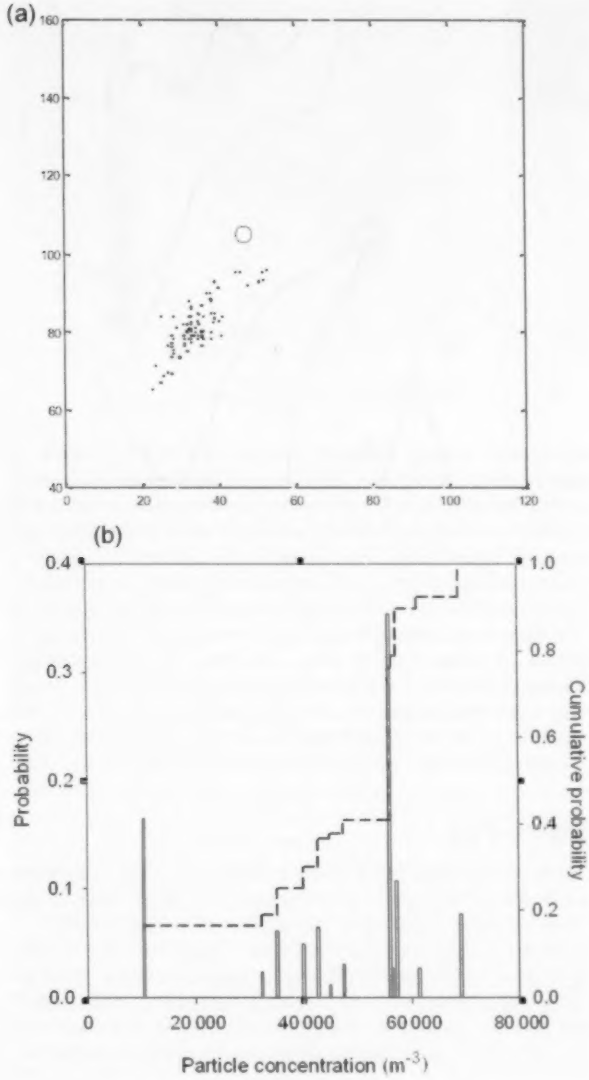
Fig. 9. The combined standardized variogram for days 200 (circle), 206 (inverted triangle), and 211 (square). The thick broken black line represents the best fit of an exponential model using nonlinear least squares. The thick dotted line represents the section of the curve for which there is no information.



part of other population studies aimed at the relationship between larval fish and their feeding environment. For example, Werner et al. (1996) use the broadscale distribution of copepods and a model of local small-scale turbulence to predict encounter and growth rates of larval fish on Georges Bank. The underlying assumption is that the variability between scales is smooth and predictable from the broadscale observations, an assumption that is neither tested nor are the implications on the variability in growth rates considered in the analysis. There is the possibility that a greater proportion of the regional variance in plankton abundance would be accounted for by the sampling strategies used in large marine ecosystems than in this study of coastal Newfoundland. However, if the interstation variance represents a significant proportion of the overall variation in plankton abundance, then sampling strategies would have to be adapted to provide information on the probability distribution of prey concentrations at subgrid scales (International Council for the Exploration of the Sea (ICES) 1993). Since the question is seldom addressed, how do we know that it is not an important limitation to our interpretation of fish–environment relationships?

The second issue facing us relates to our ability to relate change in larval growth and mortality to changes in environmental conditions, which depends on the certainty with which we can project larva–environment associations either forward or backward in space and time. The development of detailed circulation models has provided an effective tool to track drift and dispersion of planktonic organisms (Werner et al. 2001). Their spatio-temporal resolution can severely limit the accuracy of projected changes in the distribution of planktonic organisms (Helbig and Pepin 2002). Such models are adequate to resolve general circulation features and some of their variability, but turbulent motion cannot be predicted with great certainty (e.g., in the weather bandwidth of 4–15 days). This is often treated as random motion and represented as a dispersal component in most particle-tracking schemes. However, dispersal introduces uncertainty in the data–model interplay as we try to relate the state of the population to the likelihood of prey–predator interactions. An example of the challenge we have to consider can be found in the backward reconstruction of drift trajectories for particles that we consider associated with a sample of larvae from a single sampling site. In a 6-day backward projection of possible particle starting locations from the study of Trinity Bay, there is a 20-km spread of particles across the bay and ~60-km spread in the along-bay direction (Fig. 10). If these particles are associated with the nearest stations from

Fig. 10. (*a*) Backward drift simulations of the distribution of particles (dots) located within the model domain (indicated as km along the *x* and *y* axes) associated with a single sampling site where larvae were caught on day 206 (open circle) and (*b*) the relative frequency (vertical bars) and cumulative probability distribution (broken line) of microzooplankton density (counts·m^{-3}) based on the observations from the nearest station sampled on day 200.



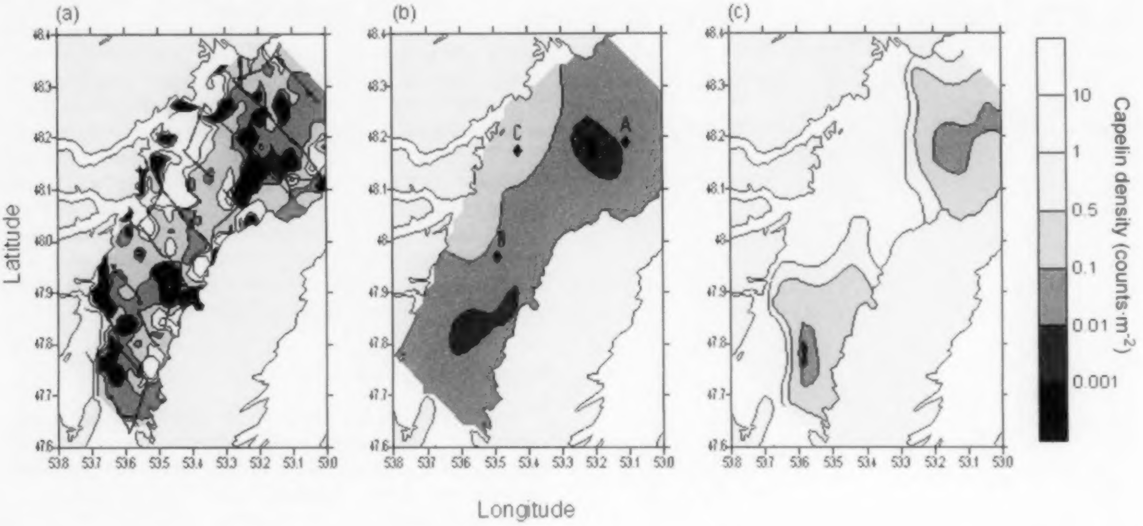
which microzooplankton samples are available (from Fig. 8), the probability distribution of microplankton densities for a single sample shows a considerable variability that is unlikely to meet assumptions about the statistical uncertainty used in conventional analytical treatments (Fig. 10). It is therefore essential that the uncertainty in our knowledge be reflected in the analysis of larva–environment interactions, which can be dealt with in several ways. One option is to explicitly model the uncertainty in our assessment of the relationship between growth and environment. However, the form of the uncertainty may not vary in a consistent manner or follow the form of a single family of error distributions, although the use of nonparametric methods may provide a useful alternative. Another solution is to use repeated stochastic resampling of the individuals and possible paths to assess the likelihood of alternate a priori models of the functional relationship between growth and environmental conditions. In both these instances, the underlying assumptions are based on a belief in the accuracy and representativeness of the environmental variability in the feeding environment of our drift projections. Their advantage is that they attempt to reflect the underlying probabilistic nature of both our knowledge and the processes that influence the dynamics of the early life history stages of fish.

Larvae as prey

To date, I have restricted my discussion to larva–prey interactions. When dealing with potential predators of larval fish, the problem becomes somewhat more complex because we have two distinct groups to deal with: invertebrate carnivores (e.g., medusae, chaetognaths, shrimp) and planktivorous fish (e.g., herring, anchovy, mackerel) (Bailey and Houde 1989). As with larval feeding, we are faced with the need to describe the combined outcome of the probabilities of encounter, attack, and escape to predict mortality rates of larvae. Of the three, the latter is probably best understood from laboratory studies, which show consistency in relation to relative prey–predator size across taxa (Paradis et al. 1996). The probability of attack is less well understood and is likely to be influenced by size, hunger, alternate prey abundance, as well as physical characteristics of the environment (Bailey and Houde 1989). However, the probability of encounter is the least well understood at this time. In most instances, encounter is treated as a random Poisson process in direct relation to average predator abundance (e.g., Cowan et al. 1999). Variation in predator aggregation and distribution are generally neglected, yet they should be represented more accurately if fisheries oceanography is to make progress in understanding the potential loss of larvae to predators (e.g., Garrison et al. 2002).

Our ability to describe or model larval encounters with predators depends to some degree on the resolution with which we can represent the predator (and prey) distributions in space. Consider the case of planktivorous fish, for which in many instances their spatial distribution can be described using hydroacoustic sampling, which allows continuous sampling over a large scale. The analysis of hydroacoustic data often involves geostatistical approaches that are well suited to stable structures (e.g., geological features) but place certain limitations when applied to mobile organisms (Rivoirard et al. 2001). Typically, data from hydroacoustic (or trawl) surveys are integrated over transect sections several kilometres in length, with this information being then interpolated using a variety of approaches (e.g., optimal interpolation, kriging). However, over a distance of ~5–10 km, which is also of the order of the estimated daily ambit for several small pelagic fish, there is considerable in-

Fig. 11. (*a*) Interpolated distribution of capelin (*Mallotus villosus*) density (counts·m^{-2}) during a survey of Trinity Bay conducted in July 2000 based on kriging, (*b*) the distribution of the median, and (*c*) scatter (range between the 10th and 90th percentiles of the cumulative probability distribution) in capelin density estimated using nonparametric local density estimators with a bandwidth of 3 km. Letter symbols in (*b*) refer to sites explored in Fig. 12.



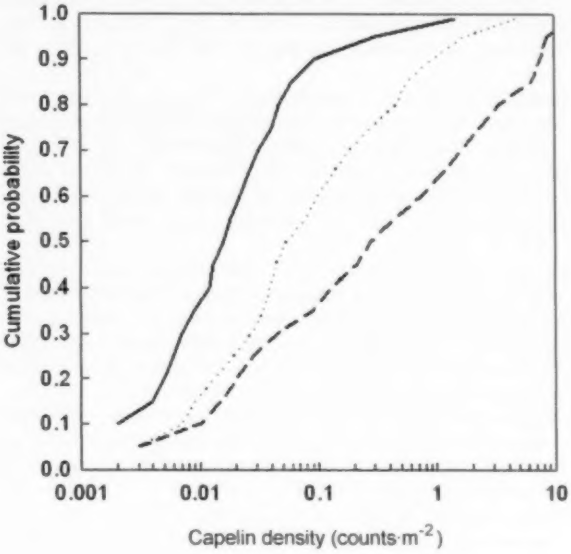
formation about the aggregation of potential predators that is being ignored. This information can provide a measure of the variation in the structure of the probability distribution of encounters between larvae and potential predators. For example, consider the results of a single survey of Trinity Bay in which we estimate the distribution of capelin based on the median hydroacoustic estimates within a 3-km radius of the 29 stations sampled in Baumann et al. (2003). The overall variation in density shows a clear spatial pattern, with high densities on the western side of the bay (Fig. 11*a*). However, if we use a two-dimensional, local density estimate of the cumulative probability distribution of capelin densities, we see that there is also a high degree of spatial structure in the underlying variability (scatter) in capelin density that does not follow that of the median (Figs. 11*b*, 11*c*). If we pick three sites across a range of medians and scatter, it becomes more apparent that the underlying form of the probability distribution of encounters (fish density) varies spatially (Fig. 12). The benefit of representing predator densities as a spatially changing probability distribution is that it is more readily reconcilable with the underlying processes of prey–predator interactions. Rather than representing the predator environment as a single, smoothly varying element (i.e., mean or median), the underlying probability of predator densities can be fully applied to estimating the probable distribution of mortality rates. Since any estimate of mortality rates is effectively a sample of the underlying processes (and error) that determine the loss of individuals, knowledge of the underlying probability distribution of encounters could provide a more appropriate basis with which to contrast our observed mortality rates.

Moving from a static (e.g., based on the mean) to a probabilistic representation of encounter rates is a first step in to providing a more effective base against which we can contrast spatial patterns in estimated mortality rates. Simple and reasonable models that represent the temporal variation in aggregation and overlap of larvae and their predators are essential because it is the variation in these elements that will result in variations in mortality rates for a given prey and predator abundance. Development of such models requires understanding of both how plankton are moved around by ocean currents and how and why mobile predators aggregate and disperse. Changes in migration timing and spatial aggregation of pelagic fishes (e.g., sardines, anchovy, herring, mackerel) have not been the focus of much research, but if prey–predator interactions are the fundamental process that determines the potential production of late stage larvae, then greater efforts have to be directed toward such issues.

Discussion

Larval fish provide us with a relatively unique organism from which to study the dynamics of marine ecosystems. They are sufficiently scare and restricted in time that we can trace their origin in space and time somewhat more readily than many other organisms (e.g., copepods). Their development and survival is a draw from a succession of probabilities of encountering prey and predators, and it is the location (mean) and form of these distributions that represent the key knowledge that we must have about the environment. Otolith microstructure provides a record of the past state of the individual with which we can attempt to infer how environmental conditions affect an individual's development. This record may enable us to infer the structure of (successful) encounters with prey by individual larvae, and changes in the distribution of growth rates among individuals within the population may give us insight about the distribution of (unsuccessful) encounters with predators. By making greater use of the probability distribution of successes (growth) and failures (mortality) and the inferences that we can make

Fig. 12. Cumulative probability distribution of capelin (*Mallotus villosus*) densities estimated at sites A (solid line), B (dotted line), and C (broken line) shown in Fig. 11.



about their occurrence in space and time, we may be able to use studies of larval fish dynamics to gain a better understanding of prey–predator interactions in marine systems.

Although the examples presented in this study are restricted in scale relative to that required for most population studies, they are intended to show that standard survey designs may fail to capture a substantial portion of the underlying variability in the distribution of the prey and predators of larval fish. The fraction of the variance in environmental conditions that is not captured by our sampling will likely vary with the tools used to characterize the system and the scale of the study site (maybe with a greater proportion of the variance being characterized in larger systems simply because of the large-scale differences that are likely to exist). To overcome this problem, one might be tempted to move to continuous sampling devices, such as towed optical plankton counters and hydroacoustic samplers, and analytical time series methods to describe the structure of the system (e.g., Lovejoy et al. 2001). However, the fine structure in the distribution of organisms is ephemeral and likely difficult to model. More importantly, sampling of the eggs and larvae would be mismatched with the scale of our view of the environment. Even if we knew the fine-scale distribution of microzooplankton between stations during a single survey of the area, how could we associate the distribution of states (condition) of the larval population at a sampling site with environmental conditions (past or present) surrounding them? What continuous samplers could provide is a view of the probability distribution of observations provided by that sampler between traditional sampling stations. This information could then be used as the basis from which tools, such as individual-based models (e.g., Werner et al. 2001), could be used to understand and forecast the state of the larval population.

There are clearly general regularities in the scale-dependent structure of marine ecosystems that vary across trophic levels (Mackas et al. 1985; Lovejoy et al. 2001). Phytoplankton with high turnover rates are more directly affected by variations in the physical forcing and show an overall structure that closely matches that of the distribution of water masses. However, as one moves up the food chain to higher trophic levels, behavior and prey–predator interactions begin to alter the randomness in the separation between organisms caused by the physical environment. There is often a degree of predictability in the distribution of marine organisms relative to the physical structure of the system, independent of trophic level (e.g., Mackas et al. 1985; Garrison et al. 2002). However, the predictability of structure (the phase component in a time series approach that appears as the underlying error in organism–environment relationships–associations) is poorly understood. As a result, the theoretical constructs used to describe changes in the biophysical environment have limited capability in forecasting changes in environmental conditions on the time scales relevant to the dynamics of early life stages of fish.

To effectively study the dynamics of larval fish, field programs have to move away from a parametric description of the environment that disregards the underlying structure in the error at scales smaller than the interstation distances. Field studies have to deal more explicitly with a description of the changing structure in the heterogeneity in the distribution of prey and predators. To do so will require changes in the current experimental design of population studies that would provide an opportunity for replication of sampling within a site (e.g., station location) in an attempt to identify changes in the underlying probability distribution of densities at subgrid scales. It is likely that environmental conditions will change the variance-to-mean relationship from one observation period to the next, so it is important to avoid adopting assumptions about the error structure with which we represent probable encounters. With traditional net sampling, one approach could be to define a number of fine resolution station grids embedded locally at a number of sites within the larger survey array. Each smaller grid would then provide a measure of the probability distribution of organisms (prey) as represented by the net samples. An alternative would be to combine the sampling of larvae using plankton nets with collections from continuous samplers (e.g., based on optical or hydroacoustic methods) to describe the aggregation patterns of organisms as represented by the traditional sampling array used to describe the distribution of larvae. Observations of prey or predator counts collected by continuous samplers could then be used to describe the probability distribution of encounters within each element (station) of the larger array. In both examples, the collections would provide a measure of the variations in both the mean and the variance in biological conditions over the study region.

Observations of physical, chemical, and biological conditions from the monitoring of marine ecosystems over long periods have provided insights about trophic relationships (e.g., Beaugrand et al. 2003), but the uncertainty in causality and the potential effect of confounding factors has constrained the long-term applicability of such findings (e.g., ICES 2000). The spatial resolution of long-term monitoring programs has remained coarse. How local prey (predator) numbers affect larval growth (mortality) and how variations in the distribution of the prey–predator complex affects overall prerecruitment survival remain among the major unknowns in fish population dynamics despite decades of research. Variations in growth and mortality rates have to be contrasted with observations of the expected distribution of encounters (and successes) with prey and predators rather than with the assumed distributions for which field collections provide a measure of the mean. Once this can be achieved successfully, short-term process studies need to address how the variation in the structure of the distribution of prey and predators can be quantified rather than assumed. The goal is to develop metrics that provide quantitative information of subgrid (interstation) variance, aggregation, and probable overlap of prey and predators, with fish eggs and larvae that could be applied to regional large-scale (monitoring) programs. If we are to understand the dynamics of marine ecosystems and apply that knowledge to minimalistic, long-term observation programs, then we must find a means to describe the structure of those ecosystems in a manner that reflects variations in the probability distributions of prey–predator interactions — it is unrealistic to expect extensive observation programs to cover all aspects of the environment of prerecruit fish.

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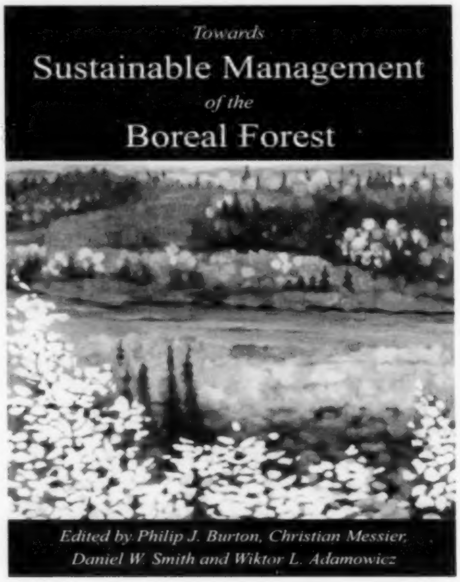
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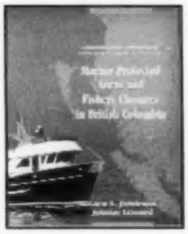

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